



March 27, 1975

The reward system needs overhauling

In this last week, and in this International Women's Year, the Royal Society marked the occasion by electing a woman as one of the thirty-two new fellows, and Fred Hoyle put the cat among the pigeons in Nobel wonderland by saying that Jocelyn Bell (now Jocelyn Burnell) ought to have got the prize for the discovery of pulsars. One can always rely on the reward system in science to breed controversy.

On the making of Fellows of the Royal Society there is, of course, no end of folk-lore, gossip and scandal. This has been true since at least the eighteenth century, when membership of the society was a fashionable thing for London gentlemen. As late as 1830 Charles Babbage could write "several times, whilst I have been consulting books or papers at Somerset House, persons have called to ask the Assistant-secretary the mode of becoming a member of the Royal Society. I should conjecture, from some of these applications, that it is not very unusual for gentlemen in the country to order their agents in London to take measures for putting them up at the Royal Society". Now the society is so purged of its non-scientific attachments that it can reasonably be accused of being too addicted to the rewarding of those who practise science and too little aware of those who teach, communicate, document, administrate and philosophise about science. It is somewhat silly that a first-class administrator with major achievements in advancing the organisation of British science should have to be admitted this year on the basis of being distinguished for scientific work which he had to give up ten years ago.

It would be invidious to name individuals whose distinction will never be rewarded under the present state of affairs (*Nature* keeps a list of fifteen or so). But it should be pointed out that the mechanism for recognising "conspicuous service to the cause of science" exists but is restricted to one extra fellow a year—at present only eleven fellows are in on that ticket, including two royals and five politicians. The mechanism also exists for "general candidates" to be admitted, although the committee charged with presenting names does not seem to have outstanding success in leavening the fellowship. By all accounts the process of selecting fellows is a parochial affair in which no external advice is sought; this may assure main-line excellence, but it doesn't recognise the new dimensions of science which represent its internal and external interactions.

In the past 30 years the representation of women in

the society has actually declined. Of those present fellows, seven were elected between 1946 and 1955, eight between 1956 and 1965 and eight in the past 10 years. In the same period the total number of new fellows elected has risen from 25 to 32 annually. The society can hardly be blamed for the shortcomings of a system in which it is still difficult for women in large numbers to pursue science with anything approaching the single-mindedness that intellectual distinction demands. On the other hand women, with every justification, are looking for some redressing of the balance. This has called in some countries for positive action by institutions, and it would be good to see the Royal Society give a lead in asking the question—how can we recognise the contribution that women make to science? More than 10% of the authors of papers in this journal are women and most of our authors are relatively young, yet by the time that they have reached an age at which they are recognised as distinguished, the proportion has dropped to about 3%.

Finally, astronomy has now had the Nobel plague descend on it. The joy of astronomers that at long last a Nobel prize is within their grasp is likely to be shortlived when they discover how divisive the whole concept of vast cash awards and hob-nobbing with royalty is to the scientific community. Fred Hoyle's complaint, however justified or unjustified, is only an early shot in a battle which will set scientists against each other in years to come as more astronomers get rewarded, and others go unrewarded.

The trouble with Nobel prizes is threefold. First, there are clearly not enough to go around, and hence there is bound to be inequity in their distribution. Second, they tend to be confined to a restricted number of subjects, giving a false impression of the relative importance of various fields. Third, they confer an unreasonable amount of credit on their recipients leading to Merton's well-documented Matthew effect ('to him that hath, it shall be given') and often to the less well-documented Boeing complex ('given the option between jetting around the world lecturing on things you don't know much about and staying at home working, go for the jet-trip any day').

In certain subjects and certain laboratories one gets the distinct impression that the pursuit of Nobel prizes is the ultimate aim, rarely spoken of but rarely very far below the surface. Ambition is a fine thing but these inequitable, divisive, flattering prizes generate obsession. Isn't it time they were abolished? □



Plutonium particles: some like them hot

The Medical Research Council's 1975 report The Toxicity of Plutonium (Nature, 253, 385) concludes that "there is no evidence that irradiation by 'hot particles' in the lung is markedly more hazardous than the same activity uniformly distributed or that the currently recommended standards for inhalation of plutonium are seriously in error." Report R29 (1974) of the National Radiological Protection Board (NRPB) states that "there is no biological evidence available at present which suggests that 'hot spots' carry a higher risk of cancer induction." But in the March 20, 1975 New Scientist, Dr A. R. Tamplin warns: "The plutonium exposure standards . . . must and will eventually be made more restrictive by a factor approaching 1,000." Behind this disagreement is a major controversy over the risk of lung cancer from inhaled insoluble particles of intensely radioactive alpha emitters such as plutonium. In that controversy—unresolved by any direct data—lies a source of uncertainty about the future of nuclear power. A report by Amory B. Lovins and Walter C. Patterson of Friends of the Earth.

SINCE the late 1960s, many experts—Geesaman, Langham, Long, Morgan among others—have expressed concern about the formidable but highly uncertain toxicity of plutonium (Pu) aerosols. On February 14, 1974, Drs Tamplin and Cochran of the Natural Resources Defense Council (NRDC) took action. They petitioned the United States authorities to reduce the maximum permissible lung burden (MPLB) and maximum permissible concentration in air (MPC_a) of insoluble Pu by 115,000 times. Also in 1974, the US Atomic Energy Commission published two defences of present standards, LA-5482-PR and WASH-1320, and in May and November NRDC published interstitial rebuttals to which the AEC declined to respond in writing or otherwise. The NRDC petition is under review by many US official bodies, and the AEC's successor agency should announce its next move in April.

The cancer risk from microscopic inhaled particles of insoluble Pu and similar actinides arises because such

particles can deposit in deep lung tissue, can stay there for a year or more, and, being hard alpha emitters, can irradiate only that tissue which is within ~40–45 μm. This last property makes it hard to determine the MPLB and MPC_a, for these are derived from an international standard which says merely that lungs should not be exposed to more than 15 rem of radiation per year. Fifteen rem is equivalent to 1.5 rad of alpha radiation (since alpha radiation is considered 10 times as carcinogenic as gamma radiation), and a rad, the basic unit of "radiation absorbed dose", is that amount of radiation which deposits 100 erg of energy per gram of tissue irradiated. But which tissue is that? Present practice is to average the alpha dose over the entire 1000-g pair of lungs whether it all receives alpha radiation or not.

This practice is illustrated in Table 1, which shows approximate data for some hypothetical distributions of one MPLB of the dominant isotope ²³⁹Pu (16 nCi = 0.016 microcurie, the amount whose

annual radiation dose, averaged over 1,000 g of lung tissue, would be 15 rem). For convenience, and with no pretence at realism, Table 1 assumes that this MPLB is uniformly distributed in the lungs with varying degrees of aggregation, ranging from a single 37-μm particle (actually too big to be respirable) to ~50 million 0.1-μm particles. But each ²³⁹PuO₂ particle, however large, can only irradiate ~50–100 of the several hundred million alveoli; therefore an MPLB uniformly distributed as particles larger than ~0.15 μm must irradiate some lung tissue at more than 15 rem per year and some other tissue not at all. (Non-uniformly distributed particles, as they would be in reality, would give non-uniform doses at even smaller particle sizes.) Nonetheless, the mean lung dose, now used to set the MPLB and MPC_a, would be 15 rem per year in each case shown, and each would be assumed to carry the same cancer risk.

The issue posed by Table 1 is whether intense irradiation of a very small mass of tissue is more carcinogenic than less intense irradiation of a proportionately larger mass; that is, if a given amount of intense irradiation is distributed more evenly over lung tissue, whether the greater number of cells at risk exactly compensates for the smaller risk per cell. An hypothesis that this were untrue would imply that carcinogenesis is not linearly proportional to dose at very high dose levels because intense local irradiation can bring to the fore some new mechanism(s) absent or unimportant at lower doses. (Loosely speaking, this is a "threshold" theory, but one applying to high doses, not low ones: people who argue for non-linearity in the low-level radiation controversy tend to argue against it in the hot-particle controversy.)

Such a high-dose mechanism might involve (for example) carcinogenic or contributory chemical activity by fragments of killed, fibrotic, liquefied, or otherwise seriously disrupted cells, rather than (or in addition to) "primary" or direct carcinogenesis by disruption of, say, a chromosome. Such a "secondary" mechanism may coexist with other mechanisms, is plausible, and has respected adherents, but has not been directly demonstrated. Neither, however, has any other proposed mechanism of radiation carcinogenesis, primary or secondary.

The International Commission on Radiological Protection (ICRP) have sometimes had to introduce a Distribution Factor into the rad-to-rem conversion to allow for inhomogeneous doses within a "critical organ". Thus the dose limit for bone is based on uniformly deposited ²²⁶Ra; but Pu, collecting at the bone surface, is considered ~5 times as radiotoxic, so a Distribution Factor of 5 is used to compute Pu bone dose. Likewise, IAEA TR-142 (1973) reports

Table 1 Some illustrative and hypothetical uniform distributions of 16 nCi (= 1 MPLB) of ²³⁹PuO₂ in 1 kg of lung tissue

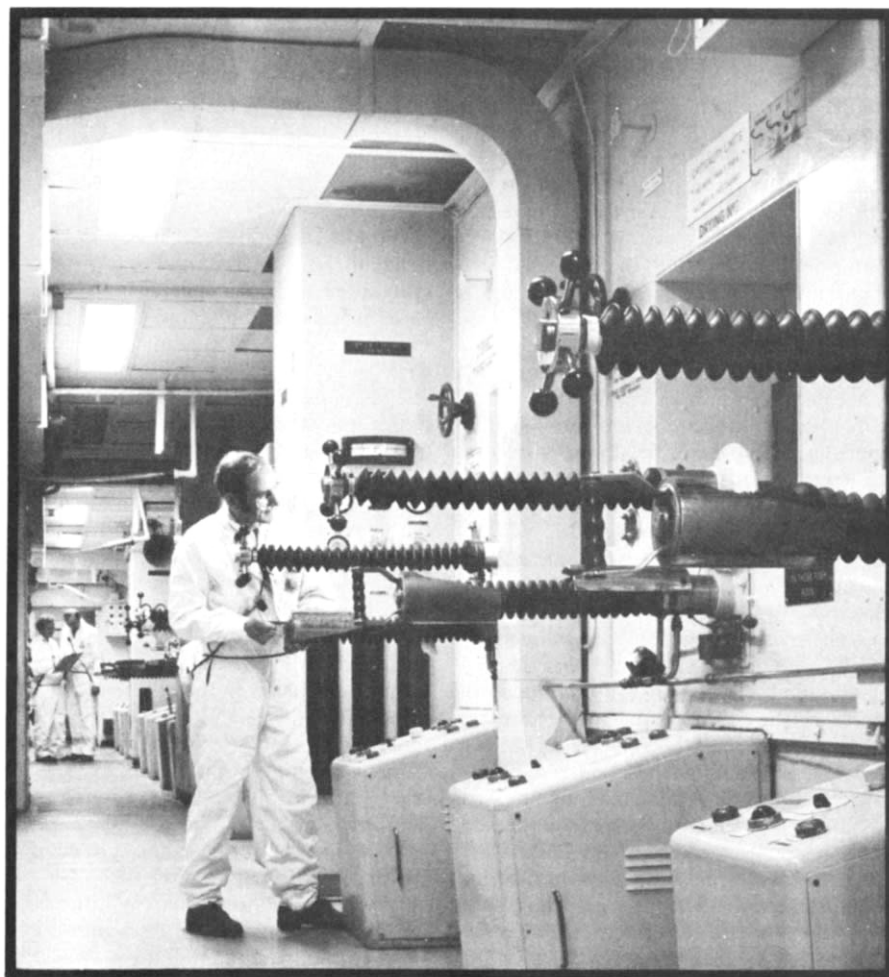
	1	50	5 × 10 ⁴	1.5 × 10 ⁷	5 × 10 ⁷
Number of particles	1	50	5 × 10 ⁴	1.5 × 10 ⁷	5 × 10 ⁷
Particle diameter (μm)	37	10	1	0.15	0.1
Particle mass (ng)	300	6	0.006	2 × 10 ⁻³	6 × 10 ⁻⁶
Particle activity (pCi)	16,000	300	0.3	0.001	3 × 10 ⁻⁴
Notional lung mass irradiated	65 μg	3.3 mg	3.3 g	1 kg	1 kg
Fraction of 1 kg	7 × 10 ⁻⁸	3.3 × 10 ⁻⁶	0.0033	1.0	1.0
Local tissue dose	200 Mrem/yr	4 Mrem/yr	4 krem/yr	15 rem/yr	15 rem/yr
Mean dose to 1 kg lung tissue	15 rem/yr	15 rem/yr	15 rem/yr	15 rem/yr	15 rem/yr

The distributions shown in the last two columns give a local-tissue dose equal to the average lung dose because the radiation fields of that many particles must overlap, whereas in the first three columns, the radiation fields from so few uniformly distributed particles are insufficient to "fill" the lung. In reality, the distribution would not be uniform, and the latter position would arise with particles smaller than shown in the last two columns. Two further points: about half the energy of the alpha radiation is dissipated in the first 20 μm of tissue radius, or in about 1/8 of the volume irradiated by each particle; and since some attenuation of the alpha radiation in a spongy lung occurs in air rather than in tissue, the mass of tissue irradiated by each particle, and hence the reciprocal of dose to that mass, would be less in compact soft tissue such as skin.

that "because it had been recognised that the dose distribution [from inhaled radon daughters] in the respiratory tract was inhomogeneous and the maximum alpha dose was delivered to the bronchial epithelium" rather than to the whole lung, the ICRP suggested a Distribution Factor of 10, owing to evidence that uranium miners were getting lung cancer more often than the average-lung-dose model had predicted. So far, no Distribution Factor > 1 has been applied to Pu in lung; and NRDC suggest, in effect, that the "critical organ" limited to 15 rem per year should be not the complete pair of lungs, but rather the tissue irradiated within it. Otherwise, using the dose-averaging concept, a Pu particle in beagle lung would be 8 times as carcinogenic as the same particle in human lung (neglecting species differences) simply because human lung is 8 times as large. Indeed, if each of two men had an identical Pu particle in his left lung but the right lung of one man had previously been removed, his computed risk of lung cancer from the particle would be twice that of the other man! This *reductio ad absurdum* shows the artificiality of averaging very localised doses over entire organs.

The ICRP have explicitly declined to offer guidance in this area: as the NRPB state, "radiological protection standards cannot be applied to small volumes as they have been derived from observation of biological effects in the whole body or in body organs." Experiments have shown, however, that intense irradiation of, or other insults to, small amounts of various tissues (including bronchial and lung tissue) can produce tumours or other lesions showing cellular changes characteristic of precancerous lesions. Indeed, Pu particles in rat lung have been shown to cause lesions similar to one observed around a 5-nCi ^{239}Pu particle excised in 1962 from a worker's palm: this lesion showed "severe" changes similar "to known precancerous epidermal cytological changes". There is strong evidence, too, associating a fatal soft-tissue sarcoma in a US worker with entry, through some minute cut, of Pu solution that had spilled on his hand. Thus cancer, or lesions suggestive of eventual cancer, is demonstrably induced in both man and laboratory animals by very intense irradiation of very small amounts of various tissues, including rat lung. But does the incidence of such effects exceed that observed for lower intensities applied to proportionately larger amounts of tissue?

No direct answer is available for inhaled insoluble Pu. Disquieting long-term experiments with beagles have shown that almost 100% of dogs inhaling Pu doses down to 200 nCi ($\sim 10^7$ particles) get lung cancer within ~ 11 years, but experiments with lower doses will not yield data for many years. The evidence



Windscale: workers on the plutonium finishing line

must therefore be indirect, so one must decide what evidence is relevant and hence exactly what is a "hot particle". The February 1974 NRDC definition, based largely on work by Geesaman, is an alpha-emitting "particle in the deep respiratory tissue of such activity as to expose the surrounding lung tissue to a dose of at least 1,000 rem in 1 year." New data (including human autopsy data) discussed by NRDC in a February 1975 paper suggest that a lower limit for hot-particle activity might be anywhere from 0.07 pCi (derived from the 1 krem per year criterion) to perhaps 4.2 pCi, —a four-fold range of particle diameter—depending on the data and degree of conservatism chosen. NRDC also prefer to define the limit not by calculated local dose but by particle activity, which can be correlated with observable phenomena: some levels of activity produce fibrotic lesions whilst others do not.

Clearly it is necessary to assume some lower activity limit distinguishing the hot-particle carcinogenic mechanism from that of uniform dose, and any such postulated limit must have a certain arbitrariness which invites challenge. (The limit may not in reality be so clearly defined.) Suggestively, the 0.07-pCi limiting activity originally proposed by NRDC is substantially above the level at which

uniformly distributed particles totalling 1 MPLB cannot irradiate the whole lung (Table 1).

No quibbling can arise over whether a particle is a hot particle if its activity is orders of magnitude too low to cause observable discrete lesions. The particles used in many experiments, those produced by fallout from nuclear testing, and those inhaled by the often-cited 25 Manhattan Project workers are all in this category and hence cannot be used to test the NRDC hypothesis. At least 25 other workers did inhale hot particles from the 1965 Rocky Flats fire, and linear extrapolation from the beagle data suggests that these men are likely to develop lung cancer after an unknown induction period, probably of several decades.

Many critics of the hot-particle hypothesis do not state whether the experiments they cite involve hot particles. For example, the NRPB's dismissive review of the NRDC hypothesis relies heavily on an unpublished paper of Lafuma, comparing the incidence of lung cancer in rats after inhalation of "particulate" ^{239}Pu or "diffuse" ^{242}Cm . NRPB claim a lower incidence for the former doses, but give no data, and one is entitled to wonder whether the ^{239}Pu was in the form of hot particles, what the doses were, whether the lifespan of the rats exceeded the

induction time of the effects sought, what allowance was made for the 23-week half-life of ^{242}Cm and so on. Inquiries to NRPB in January, 1975 elicited the astonishing reply that such basic data as particle size and activity were not in Lafuma's paper and must be sought from him. (An immediate letter to that end has had no reply at this writing.) So it is not clear whether the NRPB's crucial experiments involve hot particles at all.

Even if they do, the data are still consistent with the hot-particle hypothesis. Experiments suggesting that tumour incidence per nCi is greater with uniform than with discrete dose distribution—or the opposite, as both can be shown—are not relevant to, nor a test of, the hot-particle hypothesis: the latter bears on the risk per hot particle, not the risk per nCi. If the hypothesis is right, then increasing the activity of a hot particle—one that is already large enough to bring into play the postulated high-dose mechanism of carcinogenesis—only further disrupts already-killed cells and does not significantly increase the risk from that particle, though it reduces the apparent risk per nCi. Thus the hypothesis already contains the concept of "overkill" or "wasted radiation". Table 2 shows six hypothetical experiments devised by NRDC: *A-D* suggest that discrete dose is less hazardous than uniform dose, *E-F* suggest the opposite, and *A-F* are all consistent with the hot-particle hypothesis.

The NRDC rebuttal of WASH-1320 appears to show that all experiments there cited are either irrelevant to the hot-particle hypothesis or consistent with it. The irrelevance arises because for example, experiments do not involve insoluble hot particles in lung, or use short-half-life actinides, or show rapid lung clearance, or have been interpreted on a risk per nCi basis, or use animals that do not survive the induction period, or do not wait for it to elapse, or mask the lung-cancer response with other pathological conditions, or do not involve significant samples.

The MRC report, apparently initiated by the NRDC petition, cites it as the work of "an organisation called the Environmental Defense Research Council concerned with problems of pollution in general" (wrong on both counts) and, like the NRPB report, ignores the two 1974 NRDC rebuttals which follow up the original petition and anticipate the MRC and NRPB arguments. (The MRC report does not even cite the two AEC responses to the NRDC petition.) By failing to read these papers or to contact NRDC, the MRC and NRPB have lost an opportunity to raise arguments not already canvassed. They have stressed the quantitative uncertainties (limiting particle activity and risk per particle) but have not disproved the basic thesis. Instead, they prefer to argue that "there is no evidence" for the NRDC hypothesis—

whilst ignoring much of the evidence NRDC cite in their original paper, notably reports of Pu-induced lesions in both rats and man. But the "no evidence" argument cuts both ways. There has been no concerted effort in Britain to imitate the new US Transuranium Registry of radiation workers, which it is hoped will eventually find the epidemiological evidence if it exists. To argue from the general absence of evidence (for which one has not looked) that there is no evidence for one's opponent's point of view is to write with an economy of truth.

The main drawback of the hot-particle hypothesis seems to be that it requires one to assume an unproven and somewhat vague cellular mechanism of radiation carcinogenesis operating at high dose levels. Yet critics of the hypothesis have been unable to demonstrate any mechanism underlying their own theories, and until they have done so, it is hard to see how other mechanisms can be definitely excluded. The resulting scientific uncertainty, and its likely persistence, raise an important ethical and political issue. We have an hypothesis, neither proved nor disproved, implying that the MPLB and MPC_a for insoluble aerosols of Pu and similar actinides—inherent in a large-scale fission economy—should be reduced by orders of magnitude. We have two choices: (1) ignore the hypothesis as unproven, continue our research, continue to expose some radiation workers to hot particles, assume that these are innocuous, and proceed to make the world's energy supplies dependent on annual flows of hundreds of tons of Pu; or (2) provisionally adopt the hypothesis until it is disproven, tighten standards accordingly, and thus act with the caution, prudence, and conservatism which—we are told—are the basic principles of radiation protection. So far, regulatory bodies have taken the first course, gambling that exposed Pu workers will not begin, a decade or two hence, to show excess lung cancers as some analysts claim they are now beginning to show excess leukaemias.

The reason for this gamble may be related to a little-known fact: that even the present MPLB and MPC_a for insoluble Pu are near the limits of practical detectability, not because we lack sensitive instruments but for fundamental statistical reasons. It is not clear that present monitoring methods at, say, Windscale can demonstrate compliance even with present standards: a "puff" release from,

say, a defective glove-box may be hidden in the 24-hour integration period of air samplers, and even a real-time radiation monitor could probably not detect significant Pu levels in a typical room before most of the air in it had been inhaled or changed. If the standards became much stricter, compliance could never be shown and the standards would have no operational meaning. Whether a Pu-fuelled industry could then operate would depend on whether the burden were on it to prove compliance (impossible) or on others to prove non-compliance (presumptive but debatable) and on how strictly the relevant duties were construed.

The questions of monitoring and enforcement are not academic; they are the crux of the matter, for a few decades from now the growing gap of magnitudes between world Pu stocks (thousands of tons) and the amount which can produce a nuclear explosion (a few kg) or give a beagle lung cancer (a few μg) will require impressively and increasingly diligent care to prevent releases through accident or malice. Pu metal, nitride and carbide can be pyrophoric, burning to respirable PuO_2 ; this insoluble ceramic is itself present in advanced nuclear fuel cycles, can pass in respirable sizes through high-efficiency filters, and has contaminated dozens of Pu workers in accidents here and abroad. Pu fires have released tens or hundreds of grams of PuO_2 from US facilities and far more within them. Pu could be released by fast-reactor accidents, weapons explosions, deliberate dispersal, or chemical or microbial action on Pu-bearing wastes (like the dilute alpha wastes buried at Drigg near Windscale). Biological pathways for reconcentration of Pu—now discharged in hundreds of Ci per year from Windscale into the Irish Sea—may exceed those now known, as Pu chemistry is very complex. Since the half-life of ^{239}Pu is 24,390 years, and since fallen-out aerosols may become resuspended (data on this vary over 11 orders of magnitude), dispersal may be essentially permanent. In an energy economy based on hundreds, then thousands, of fast breeder reactors, each containing several tons of Pu, even if Pu escape were controlled with the most exquisite care, the unavoidable limits of *in vivo* and environmental measurement would make it impossible to tell whether we were meeting stricter Pu standards or not. We could only tell from increased lung cancers—decades after the irrecoverable releases had occurred. □

Table 2 Some hypothetical hot-particle experiments

Experiment	A	B	C	D	E	F
Number of hot particles	6000	4000	2000	200	6000	4000
Total activity (nCi)	12	12	12	12	12	6
pCi/particle	2	3	6	60	2	1.5
Number of tumours observed	3	2	1	0	3	2
Tumours/nCi	0.25	0.17	0.08	0.00	0.25	0.33
Tumours/particle	0.0005	0.0005	0.0005	—	0.0005	0.0005

international news

THE dramatic revelation that the Central Intelligence Agency (CIA) last year secretly salvaged part of a Soviet missile-carrying submarine from the bottom of the Pacific, could hold some important implications for oceanographic research. Though such concerns are dwarfed by the wider political ramifications of the affair, some knowledgeable observers last week pointed out that the CIA's salvage job could affect the outcome of the Law of the Sea Conference which is now grappling with, among other things, issues concerned with freedom to carry out research in, on and under the high seas. Moreover, the affair could upset the flourishing co-operation between the United States and the Soviet Union on oceanographic research.

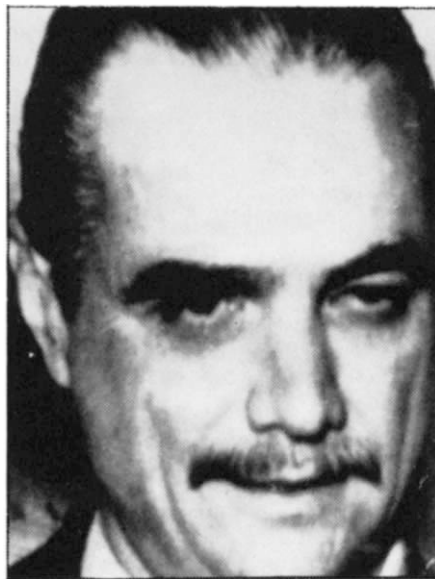
The operation had all the essential ingredients of a James Bond thriller, involving as it did a national intelligence operation, an elusive billionaire, an elaborate cover, and hundreds of millions of dollars. For five years it was also one of the Nixon and Ford Administration's best kept secrets, until a number of newspapers last week decided to print the details, in spite of frantic attempts by the CIA to keep it bottled up.

Known as Project Jennifer, the operation began to take shape soon after the submarine sank in about 16,000 feet of water in 1968, about 750 miles north-west of Hawaii. Intelligence sources in the United States knew the exact location of the vessel because they were able to pick up underwater explosions from it, but Soviet ships looked for it in the wrong area according to a report in the *New York Times*. When the Soviet ships abandoned their search, the CIA decided to try a salvage job itself.

The cover for Project Jennifer was inspired, to say the least. A special salvage ship was constructed for the CIA by Summa Corporation—a huge industrial concern operated and owned by Howard Hughes, the eccentric and elusive billionaire—and it was publicly billed as being the biggest seabed mining vessel ever constructed. Another essential component of the operation, a massive submersible barge, was built by the Lockheed Corporation; it was also depicted as playing a central role in bringing manganese nodules to the surface, but its sole function was to hide the submarine when it was recovered.

'Jennifer' subterfuge scuppered

by Colin Norman, Washington



Hughes: obsessively secretive

Because the nascent technology of seabed mining was jealously guarded in the early 1970s by the few companies hoping to scoop manganese nodules from the ocean floor, and because Hughes has a reputation for obsessive secrecy, few questions were raised about the clandestine nature of the operation. Moreover, the submarine went down in an area of the Pacific where manganese nodules are known to litter the seabed.

The salvage ship, known as the Hughes Glomar Explorer, was launched in 1973, and it made its first salvage attempt in July and August last year. According to reports, it succeeded in lifting the submarine from the sea bed, but recovered only a part of it. Although some of the accounts are conflicting, it is widely believed that the CIA failed to retrieve the two chief items it wanted—the nuclear-tipped missiles and code machines—and another attempt was being planned for later this year. But publication of the details has, apparently, scuppered that plan.

As far as the implications for re-

search are concerned, one important aspect of the affair is that the Glomar Explorer was operated by Global Marine Inc., a company which has built, owns and operates a number of oceanographic research vessels. Prominent among its fleet is the Glomar Challenger, the highly successful drilling vessel which is operated under contract to the National Science Foundation, and which has been conducting the six-year-old Deep Sea Drilling Project (DSDP).

Last year, the Soviet Union joined the DSDP, and it is now contributing \$1 million a year to the project. Although the Soviet government had made no comment on the CIA's salvage job by the end of last week, one US official connected with the DSDP speculated privately that the Soviet Union may now reconsider its participation, in view of Global Marine's connection with the CIA.

Moreover, one newspaper account even suggested that the Glomar Challenger had played a direct role in the initial reconnaissance for Project Jennifer, but that report was hotly denied by the National Science Foundation (NSF). For one thing, as the vessel's publicly available logs show, it was not in the vicinity of the submarine at the time, and for another, two Soviet Academicians were aboard when it was alleged to be scouting out the wreck. An NSF spokesman also pointed out that the Glomar Challenger has been operated continuously under contract to the foundation since it was launched in 1968, and at no time could it have been diverted to the CIA's operation.

As for the Law of the Sea Conference, one issue to be resolved is the extent to which oceanographic research should be allowed to take place unhampered both on the high seas and also within the 200-mile 'economic zones' which may be established around the coasts of maritime states. The nub of the issue is that most industrialised nations are arguing for scientific research to be permitted unimpeded outside territorial waters. But the developing nations have generally argued that research operations could provide a cover for economic exploitation of seabed minerals, and for espionage. The CIA's cover for Project Jennifer provides all the justification in the world for such fears.

The point has not been lost on delegates attending the Law of the Sea

Conference which, ironically, reconvened last week in Geneva. According to another *New York Times* account, Christopher W. Pinto, a delegate from Sri Lanka and a leading spokesman for the developing nations, has already said that the CIA operation has undercut the argument for freedom for research. He is quoted as saying that the developing nations have been suspicious that research activities may provide a cover for other operations, and "now that this is confirmed, they [the developing countries] can be more forceful".

Another aspect of the salvage operation is important for the development of undersea technology: by depicting the Glomar Explorer as a deepsea mining vessel, the CIA provided the greatest possible incentive for other mining companies to step up their operations. When the ship was being built and operated, it was generally assumed that Hughes was leading the field in the race to scoop manganese nodules from the deep sea bed.

During Congressional hearings in 1973 and 1974, for example, representatives from other prospective seabed mining companies argued vigorously for Congress to pass legislation which would offer them some financial security so that they could raise and invest vast amounts of capital in the technology. Although Congress did not pass the legislation, there is little doubt that the prospect of Hughes beating the rest of the industry to the seabed riches stimulated frenzied efforts in other companies.

It is unclear, however, whether or not the Glomar Explorer could be used for seabed mining. It is also unclear who would own and operate it for such activities, although a good case could be made out for the argument that it belongs to the federal government.

As a footnote to the operation, it should be pointed out that Project Jennifer reportedly cost about \$350 million, which is considerably more than the federal government's entire budget for oceanography for the past five years. The money was, however, provided with the knowledge of very few Members of Congress and of only a few people in the Administration. □

Call for cuts in astronomers

by Colin Norman, Washington

A COMMITTEE of the National Academy of Sciences this week made the painful and perhaps unprecedented recommendation that graduate departments in the United States should reduce their output of astronomers because of dismal employment prospects and sagging financial support for astronomy.

Although other groups of scientists have urged that production of PhDs in some fields should level off, few have openly called for graduate education to be cut back. But the situation in astronomy is considered so serious that "we can't just sit and wail about it", Dr Leo Goldberg, Director of the Kitt Peak Observatory and chairman of the Academy committee, said in an interview last week. He added that even if enrolment in PhD courses in astronomy and astrophysics were cut in half, there would still be too many astronomers chasing too few jobs.

The problem is familiar enough. Force-fed by direct government support and by the burgeoning space programme in the early 1960s, astronomy experienced a period of spectacular growth, and graduate departments expanded rapidly. But, just as large numbers of astronomers began to emerge from the universities with freshly minted PhDs in the late 1960s

and early 1970s, government support slackened and job opportunities rapidly dried up. And the situation is getting worse because, unlike most other fields of science, graduate enrolment in astronomy is continuing to increase so that the supply and demand for young astronomers is going to get further and further out of balance.

To put the matter in perspective, Goldberg suggested that employment is likely to be available for only about 50 new astronomers a year, but last year alone the universities awarded more than 180 PhDs in astronomy and astrophysics. Adding to the problem is the fact that many people with doctorates in other branches of physics will be chasing jobs in astronomy and moreover, there are now between 150 and 200 astronomers holding temporary postdoctoral appointments who have no prospects of moving on to full-time employment, Goldberg said.

The growth in university astronomy departments may reflect the fact that the science continues to be intellectually exciting. But these days, intellectual excitement does not guarantee government support, particularly when large capital expenditures are constantly required.

According to figures gathered by the committee, funding for astronomy peaked in 1968 at about \$227 million, and by 1972 (the latest year for which figures were available) it had shrunk to \$187 million. And there are scant prospects of significant growth in the next few years, particularly in space astronomy. Recently, for example, the space shuttle has been taking up a growing share of NASA's stagnant budget and there is every reason to expect the trend to continue.

In view of the prospects of expanding graduate enrolment in astronomy and shrinking financial support for the science, the committee has suggested a number of controversial remedies.

- Most important, the rate of production of astronomers should be reduced, and "it is the responsibility of every university department which produces PhDs with specialisations in astronomy and astrophysics to assist in achieving this reduction", the committee states. All PhD students should be informed of the employment situation before embarking on graduate work, they should be carefully screened during the early years of their studies and the weaker ones should be weeded out. Goldberg also suggested that a limit should be placed on the number of universities offering PhDs in astronomy and astrophysics, so that as new departments are set up, older and less productive ones are phased out.

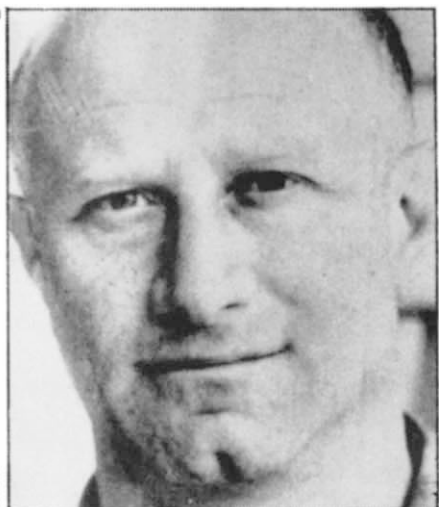
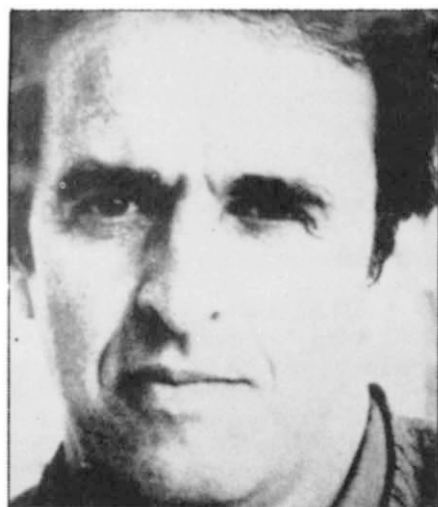
- To make astronomers more employable in other fields, the committee recommends that PhD astronomy

BRITAIN'S nuclear power industry has been further streamlined with the announcement last week of the merger of Britain's two nuclear power groupings, British Nuclear Design and Construction (BNDC) and the Nuclear Power Group (TNPG), into one company, the Nuclear Power Company (NPC), which has been acquired by the National Nuclear Corporation (NNC). The government has agreed to make an *ex gratia* payment of up to £1.416 million to the shareholders of the now defunct companies for "unrecovered expenditure". Announcing the payment, Secretary of State for Energy Mr Varley said that the acquisition of the two consortia with their staffs by the NNC was "an important step forward". The acquisition includes the govern-

ment's 26% stake in British Nuclear Design.

The shareholding structure of the NPC and the names of the chief executives will be announced in the next few weeks, and it is expected that GEC, who were in favour of building the light water reactors rejected by the government last year, will drop its shareholding to 30% from the present 50%, with the UK Atomic Energy Authority increasing its stake from 15% to 30%.

The two existing bases, at Risley, Cheshire, where the design for the steam generating heavy water reactor is being carried out and Whetstone, Leicestershire, where work on the last two advanced gas-cooled reactors is continuing, are being retained for the "foreseeable future".



REPORTS from Moscow indicate that a new campaign is in preparation against the illicit "Sunday seminars" of *refusenik* scientists. Since the departure for Israel of Professor Aleksandr Voronel, founder of the seminar, the group has continued to meet, the venue alternating between the apartments of three leading members: mathematician Aleksandr Lunts, and physicists Viktor Brailovskii and Mark Azbel.

Of these three, Lunts has been threatened by the KGB with a show trial under Article 64 of the Soviet Penal Code, which relates to treason, which could result in a death sentence. The charge, phrased in deliberately vague terms, being that he had "undertaken a task on behalf of someone outside the Soviet Union for monetary gain".

Brailovskii, while not directly threatened, was called in for a discussion with Investigator Aleksandrov, who informed him of the charges being prepared against two other dissidents, dentist Mark Nashpits and engineer Boris Tsitlioni.

Mark Azbel, so far, does not seem to have attracted the attention of the authorities to the same degree, although there has been the usual routine harassment, and being a theoretical physicist, he is making a determined effort to continue his academic work, although cut off from all libraries and other facilities. The events of the last few years, however—arrests, hunger-strike and the

nervous stress caused by constant uncertainty—has resulted in considerable deterioration of his health (he is suffering from severe cholelithiasis with reflex attacks of angina pectoris), and any increased pressure from the authorities could have very serious consequences.

● A DECREE of the Central Committee of the Communist Party of the Soviet Union (CPSU), published on March 21, 1975, states that the Jubilee Meeting of the Academy of Sciences of the USSR will be held in October 1975.

This meeting, originally scheduled for May 1974, was postponed, ostensibly on the grounds that further preparatory work was needed, although, at the time, the decision to cancel the planned international gathering was widely assumed to be connected with the concern expressed by Western scientists about the lack of academic freedom in the USSR and, in particular, the plight of Soviet Jewish scientists wishing to emigrate to Israel. This assumption was reinforced by the notice of postponement, which suggested that the celebrations would now be aimed at the grass roots of Soviet society.

The text of the new decree, after commenting on the successful local celebrations held throughout the Soviet Union on the theme "The achievements of science are for the national economy", states that the Jubilee Session of the Academy will be held

"in Moscow, in October this year, with the participation of a Party, Soviet and community organisations, representatives of workers, scientists from the Academies of Science of the [constituent] republics, the Academy of Medical Sciences of the USSR, the Lenin All-Union Academy of Agricultural Sciences and the Academy of Pedagogic Sciences of the USSR. The Academy of Sciences of the USSR is given the task, together with the Jubilee Committee, of working out and presenting to the Central Committee of the CPSU proposals connected with the accomplishment of the celebrations of the Jubilee of the Academy of Sciences of the USSR.

"The Central Committee of the CPSU expresses its assurance that the Jubilee Session of the Academy of Sciences of the USSR in honour of the 250th anniversary of its foundation will be interpreted as a nation-wide festival, and will be conducted under the badge of a review of the achievements of Soviet science and the mobilisation of scientific teams for the solution of the problems proposed by the Twenty-fourth Party Congress on the acceleration of the rates of scientific and technological progress, ensuring the economic might of our homeland, strengthening the defensive potential of the country, raising the material prosperity of the workers, consolidating peace, and strengthening friendship between nations."

courses should be broadened to include experience in teaching, computer applications and electronics. "It is up to the entire community of astronomers to correct the image that astronomers are able only to look through a telescope and ponder the riddles of the universe," the committee adds.

● To help create more jobs for astronomers, the committee recommends that undergraduate astronomy

programmes should be expanded. At present, the bulk of the astronomy community is employed in departments which award PhDs, and very few are to be found in undergraduate departments and colleges; the expansion of undergraduate astronomy courses would therefore help open up teaching posts. Coupled with that suggestion, the committee recommends that astronomy centres should establish arrangements for carrying out collaborative projects

with astronomers in more 'isolated' research environments—such as undergraduate departments and liberal arts colleges.

Although there is considerable financial incentive for universities to carry on expanding their postgraduate departments, Goldberg said he has "considerable optimism that the recommendations will be taken up" and he pointed out that Harvard has already cut enrolment in astronomy PhD courses. □

Sweden's nuclear power game

from Wendy Barnaby

SWEDEN'S nuclear future has been decided—officially until 1985. The ruling Social Democrats' energy plan, put to Parliament last week and expected to be approved, provides for the construction of nine reactors. With the four already in operation, Sweden will have thirteen reactors with a total electrical generating capacity of about 9,500 MW by 1985. This will be only two more than previously planned, but the increase is seen by critics as the thin end whose wedge will be an existing government plan to build 24 reactors by 1990. Construction on that scale would make the Swedes the world's greatest per capita users of nuclear power.

The plan is a piece of politics worthy of Prime Minister Olof Palme's considerable skills. Forced last year by public concern over the nuclear programme to postpone a decision on those plants not then under construction, he has with the present proposal given the appearance of responsible caution while allowing his government a reconsideration in 1978 of the ways energy is to be provided for 1985–90. By 1978, he explained, there would be more knowledge available on which to make a better-informed decision. By 1978, he could have added, the next parliamentary election will be safely out of the way with the emotional energy issue securely pegged for future consideration.

On the face of it, nuclear power seems an obvious choice for Sweden. Although the country has no cheap uranium resources (that is, uranium which can be mined for less than \$10 a pound), it has almost half the non-communist world's known supplies of uranium in the next price range—\$10–15 a pound. It has been estimated that the supplies of cheap uranium may become scarce in the mid-1980s, partly because of the eight-year time lapse between discovery and production of deposits. Unless exploration is stepped up, therefore, the more expensive uranium could come into demand at about the same time as the Swedes' suspended nuclear programme would have been—and perhaps will still be—well advanced. Sweden would expect to be a major exporter of reactor fuel elements. But the nuclear issue has caused an extraordinarily vigorous public debate over the past year, focused mainly on the safety aspects and the disposal of nuclear waste. (In fact Sweden neatly solves its radioactive waste problems by sending its reactor fuel elements to Windscale, England, for reprocessing.) More recently the social and security consequences of a nuclear

decision have come into focus. Pro-nuclear groups have insisted that no rise in the standard of living will be possible without large scale nuclear power, and have pointed out the dangers of dependence on foreign energy sources. Anti-nuclear forces have responded that the choice is not between increased growth and alternative energy sources: both are possible.

The two new reactors proposed by the government are officially justified on the grounds that they, together with increased hydroelectric power, will make up the extra 15 billion kWh of electricity necessary for a projected increase of 2% a year in energy consumption. Non-oil-fired power stations are being emphasised in an attempt to lessen the country's dependence on the Middle East. As the average annual increase in consumption over the 15 years until 1973 was 4.5%, the new projection will certainly test the efficacy of save-energy campaigns. It is hoped that, by 1990, growth in energy consumption will be zero.

As well as changing the relative emphasis on present energy sources, the plan provides money for research into fusion, geothermal, wind and solar power. But the fact that the allocations for this research are roughly only 10% of those for the building of new reactors shows where the government's confidence lies. It will be surprising if, having laid the groundwork so skilfully, the Social Democrats do not use the 1978 review to hasten the day when the Swedes will be the largest users of nuclear energy in the world. □

OECD energy

THE Organisation for Economic Co-operation and Development (OECD) report on the problems and perspectives of energy research and development published earlier this year provides a comprehensive and relatively up to date (September 1974) review of expenditure on all facets of energy research throughout the OECD countries, which include Europe, the United States, Canada, Australia, New Zealand and Japan.

Although the report does not commit itself to specific criticisms of member states' energy research programmes, it obviously feels the need for a longer term approach to the problem, which appears to be lacking in many aspects of energy research programmes set up in response to the 'energy crisis' of 1973. It warns for instance that the sudden unsurge in energy R&D must not be subjected to cutbacks once the most spectacular effects of the crisis have receded and the problem has become less sensitive politically.

In general the distribution of resources should be directed to keeping

as many alternative sources of energy open as possible. Research aimed at energy production should no longer be limited to one primary source. Perhaps the country which has made the largest turnaround is the United States which now supports a massive and diverse programme on every conceivable aspect of energy production but which previously had based its energy policy very largely on the availability of cheap imported oil.

One important and as yet relatively undeveloped field of energy research is energy systems. This covers the inter-relationships between production, transport and use of energy and also takes in any factors which might have a bearing on the smooth functioning of that system, such as effects on the environment and supply of skilled manpower. The use of energy accounting to clarify the energy flow through these systems from the level of primary energy up to the finished product is also encouraged. The study of energy systems, says OECD, can be an extremely important factor in moulding future policies.

Although the tables of statistics and research programmes are necessarily incomplete and sometimes countries cannot be directly compared as some figures include a measure of industrial research whereas some pertain to government expenditure only, they provide interesting reading. The USA of course leads the field spending over \$1,000 million in the fiscal year 1974. France and Germany spent around \$350 million and \$450 million respectively and the United Kingdom spent around \$228 million in 1973–74.

With regard to the organisation of energy policy, the OECD picks out Britain and the United States as the only two countries which have set up a new ministry or agency to deal with the complete problem, thus fixing a policy course that links energy R&D administration with general energy policies. In other countries, energy research has been taken under the wing of the science ministries, where they exist, or handed to some *ad hoc* committee.

Where energy problems can be reduced to matters of technology to be solved by a specific research programme, the outlook seems fairly optimistic. The member states of OECD, comprising as they do the most highly developed industrialised societies, are orientated to cope relatively easily with that type of problem. But energy is an extremely delicate political subject and affects the whole nature of society. Therefore, says OECD, "future development [of energy] will mainly depend on the political decisions of countries with regard to the nature of their economic growth and social structures". □

The decision made last year that Britain's next commercial reactors should be of the steam generating heavy water type (SGHWRs) has given a predictable filip to morale at the United Kingdom Atomic Energy Authority's research establishment at Winfrith, Dorset, where the 100-MW prototype has been supplying electricity to the grid for more than seven years. And not suprisingly, a greater proportion of Winfrith's annual spending of £9 million (of which £5 million represents payment for the electricity Winfrith supplies to the national grid) is being channelled into work on the SGHWR, at the expense of some of the establishment's other activities.

The Winfrith SGHWR has the rare distinction of being based on a design for a reactor more than five times its size, which means that scaling up to a commercial reactor of perhaps 660 MW (a size which matches the capacity of the electricity generating equipment now widely used in Britain) should be child's play by comparison with the same exercise for the British advanced gas cooled reactors (AGRs); the AGR programme in Britain is hopelessly behind time and still far from complete.

The secret is that an SGHWR is really a collection of mini-reactors of about 1 MW strung together. As the diagram shows, the basic building block, a pressure tube assembly, is remarkably simple: water at high pressure enters at the bottom and emerges from the top as steam which is fed to the turbines. Put 104 of them together in parallel and you have the Winfrith reactor, or a reactor of almost any desired size by varying the number.

Although Winfrith will continue to be interested in the kind of research that is best carried out on a prototype reactor—testing of new component designs and materials, for example—it will be for the Nuclear Power Company to finalise the design details for the first commercial SGHWR.

Presumably at that time, too, people will start to talk again about the prospects for exporting SGHWRs. Although the potential customers of a few years ago, such as Australia, Greece and Finland, are probably lost, it is at least now possible to say as part of a sales pitch that electricity utilities in Britain are also buying them.

● MORE than a year since energy policy became a hot parliamentary property, the British Secretary of State for Energy, Eric Varley, continues the tradition of the committees under his purview by appearing before Commons sub-committees, armed with good intentions. When he first appeared before the Energy Resources Sub-committee of the Select Committee on Science and

Technology, Mr Varley said last year that since he had only been at the job for three months it was early days for him to be formulating hard and fast policy. But he promised he would do his swotting up while listening to all kinds of advice. A little later his Advisory Council on Energy Conservation came before the same sub-committee and said that they too were open to suggestions, though they had precious

about suggesting what could be done, said Brown, naming Ieuan Maddock (Chief Scientist at the Department of Industry) as the "godfather" of the gang. Mr Brown asked whether or not Mr Varley had the right people on the job (the energy conservation group are in fact part-timers) and the committee chairman, Arthur Palmer, confessed he had the distinct impression that Chief Scientist Marshall had too much on his plate, what with running Harwell and sitting on all those committees.

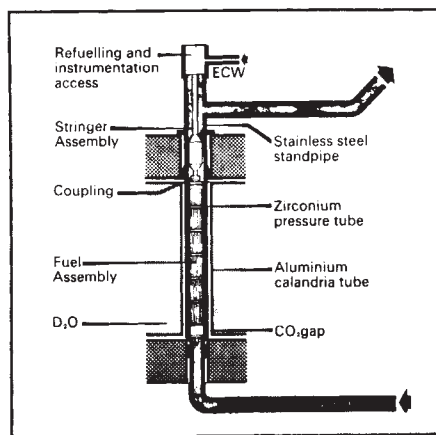
Not so, replied the Secretary of State, stoutly. Marshall wasn't complaining about overwork, and the advisory boards were manned by chaps with enormous experience. If they didn't appear to be pulling up trees it was worth bearing in mind that only 1% of energy used in the country was under government control, the rest being used by private industries which the government could simply advise. Alas, Mr Varley was leading with his chin. Apparently, one of the Department's advisory groups, the Advisory Council for Research and Development of Fuel and Power (ACORD) has been busying itself with the energy problems of the nationalised industries only, when its brief placed no such restriction on its activities.

When this was pointed out to Mr Varley, along with a suggestion that it was unrealistic to plan national energy policy by investigating only the nationalised concerns, the Secretary of State looked moderately sick and conceded that it was "a very good point" which had not been brought home to him before, and which he would look into.

As for the statistics, which caused him to brighten, at the outset, Mr Varley reported oil imports running recently at a yearly rate of less than 100 million tons, with the likelihood of even lower levels to come. This figure compared with 112 million tons in 1973 and 109 millions tons in 1974. Total energy consumption in 1974 was 4.5% down on the previous year and inland deliveries of oil in January this year were 4% down on the figure for 1974 and nearly 13% less than for January 1973. Petrol consumption, which normally rises at an annual rate of 7% was 5% down last January on the figure for January two years earlier.

But the committee wanted to know how much of this cut-back was positively caused by implementation of the Department of Energy's fuel-saving policy, and how much was caused by the general industrial recession. Not possible to say, claimed Mr Varley, but he promised to have somebody make the attempt. Certainly it can't all have been caused by a sudden overstocking of refrigerators.

Energy in Britain



SGHWR boiling channel

few of their own to offer. Then followed, earlier this year, Varley's new Chief Scientist, Harwell *wunderkind* Walter Marshall, who virtually burst at the seams with enthusiasm while regretting that it was yet, alas, too soon to be expecting any energy blueprint.

Now Mr Varley has made a return appearance before the Commons energy group, who left him with what one Member of Parliament described as a flea in his ear. Which is to say they gave him to understand that they were not entirely bowled over by the progress of the Labour government's energy strategy. Reviewing the state of the game, Mr Varley harped on his December campaign of exhortation once too often for Labour member Mr Ronald Brown. The 'Save It' campaign was 40-year-old thinking, Mr Brown suggested. And the Energy Secretary's plea that people should economise by keeping their refrigerators three-quarters full was "pedestrian".

Mr Brown went so far as to suggest that the energy chief was in thrall to bodies of important chaps who were so busy beaver away at their unco-ordinated committees that the whole energy package was turning out to be a "hotch potch". The chosen big-wigs were generally firm about the things which could not be done, but less firm

correspondence

More on nutrition

SIR,—Since I participated in what Rivers says may have been the “golden age” of nutrition, I feel that I should comment on his indictment (January 10). He says that since 1945, “nutrition in the UK has been neither of military importance nor Nobel Prize ranking.” The first circumstance is difficult to deplore since it mainly results from the lack of World War III; the second criticism is tinged with nostalgia. Rivers apparently expects nutritionists to produce miraculous “cures” of “obesity, diabetes mellitus, kwashiorkor, ischaemic heart disease and many of the other great nutritional [sic] scourges.” He suggests that the reason that these cures have not appeared is the lack of an experimental animal with an evolutionary defect such as the loss of L-gulonolactone oxidase in the guinea pig. In other words, he thinks that these “scourges” are deficiency diseases awaiting the discovery of an ascorbic acid. But much obesity results from over-eating, only occasionally aggravated by endocrine imbalance; diabetes mellitus comes from hormonal insufficiency; and kwashiorkor from economic, cultural and environmental deprivation. Whether ischaemic heart disease is a nutritional deficiency is not known.

Rivers says that “we do not know why nutrition education fails when advertising works”. But, alas, we do and this sentence is the keynote to Rivers’ lack of understanding of the predicament of nutrition. This is the day of the nutritional pied piper. The story of scientific nutrition is too humdrum to attract popular attention, at least in the USA, in the fierce light of competition from the writings and speeches of Adelle Davis, Carlton Fredericks, Linus Pauling, Jerome Rodale, Doctor Atkins, R. J. Williams and the brothers Shute who promise relief from heart disease, cancer, infections, prostate trouble, obesity, shortness of life, and back pains by devouring pumpkin seeds, raw milk, dried seaweed, ascorbic acid, fertilised eggs, apricot seeds, vitamin E and garlic, or by not consuming white bread, pasteurised milk and food additives. As Philip White of the American Medical Association says, “what usually happens is that the scientific spokesman ends up sounding like a close-minded Establishment bore who wants to suppress bright new ideas”.

Nutritionists wearily spend their time trying to pick up the pieces in the wake of this ideological hurricane. Yes, we know too well that advertising works. Rivers kindly informs us that we “are not in business to pursue uncluttered academic research” (where have scientists heard this before and how does Rivers expect a remedy for ischaemic heart disease without research?) “but to improve the nutritional status of the population”, to do which we “don’t know how”. It sounds as if he doesn’t know what he is talking about. One thing we do not need is more Nobel laureates, especially loquacious ones. We have the tremendous task of applying the knowledge of nutrition to the needs of a species that is fantastically eccentric and variable in its attitude towards the acceptability of food, and that is increasingly influenced by nutritional nonsense dispensed by the mass media. The task is made frustrating by the fact that the annual bulk cost for supplying the needed allowances for ten vitamins and eight essential minerals is less than \$1 per capita, but for socio-economic political and logistic reasons, we cannot get them to most people. On top of this, we are faced by the awesome world food crisis. There is little helpfulness in Rivers’s fuzzy sermonising. There is need for calories and there is no alternative to photosynthesis.

THOMAS H. JUKES

Berkeley, California

Peaceful explosions

SIR, — That the Non-Proliferation Treaty has defects, to some of which you have drawn attention, is widely accepted, but to adapt a well-known phrase, it is the best treaty we have. Roughly two-thirds of UN states are full parties to the treaty, which requires states to refrain from policies many of them would otherwise actively pursue.

Your particular complaint that nothing has been done to implement the requirements of Article V, that there should be an international organisation for the provision of peaceful nuclear explosions to non-nuclear weapon states, is not strictly correct. On the recommendation of the Conference of Non-Nuclear Weapon States in 1968, the UN General Assembly agreed in 1971 that the International Atomic Energy Agency itself should assume that responsibility. So far Czechoslovakia, Madagascar and Romania have enquired about services,

but a specific proposal is yet to come.

This does not mean that peaceful nuclear explosions will not be a contentious issue at the Review Conference in May, but the issues will be different from those you suggest. Now that Congress has for practical purposes put an end to the Plowshare programme, and as it becomes clearer that the Russian programme of peaceful explosions, however extensive, is yielding disappointing results, the nuclear powers will be tempted to suggest that non-nuclear parties should forego peaceful explosions. Politically that would be dangerous; let non-nuclear states discover economic reality for themselves.

That the treaty is for practical purposes innocent of security assurances for non-nuclear weapon states is true enough, as your leading article says, but it could hardly be otherwise. In practice, the best way of achieving this goal is along the lines of the Latin-American denuclearisation treaty (the Treaty of Tlatelolco, 1967). Indeed, there is good evidence that states unwilling to accede to the NPT will take part in regional arrangements like this. Although the main defect of the treaty is, as you say, that it asks for “minimal concessions” from the nuclear powers, it does require of them rapid progress towards a comprehensive test-ban treaty and measures for the reduction of nuclear stockpiles. Indeed, the NPT is a compact between non-nuclear states and nuclear states in which the former undertake to refrain from “horizontal” proliferation and the latter to pursue nuclear arms control. Much of the argument at the review conference will no doubt hinge on the extent to which the superpowers can fob off their critics with the Threshold Test-Ban agreement and SALT II. Probably they will not succeed. There will be pressure for more effective means of monitoring the super-powers than five-year reviews provide.

Precisely for this reason, the NPT is a potentially important forum within which measures of nuclear arms control can be developed. At the same time, there are many significant ways in which the inequalities of the treaty can be softened, not necessarily within its legal structure. To “suggest the treaty be scrapped and new approaches tried” is to run the risk of setting back arms control for a decade or more.

JOHN MADDOX

London EC4, UK

news and views

Excitement over β_2 -microglobulin

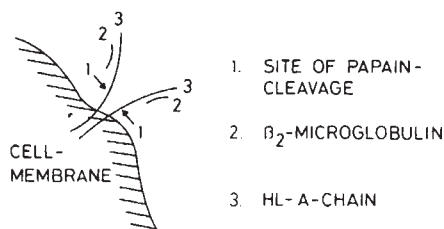
from Martin C. Raff

FROM its meagre beginnings in 1968 when it was isolated from the urine of patients with renal tubule dysfunction (Berggård and Bearn, *J. biol. Chem.*, **243**, 4095), β_2 -microglobulin has rapidly blossomed into a molecule of great interest and importance to immunologists. It is synthesised and secreted by most nucleated cells of vertebrates (Nilsson *et al.*, *Nature new Biol.*, **244**, 44; 1973) and is also present on the surface of these cells, as well as in the urine, serum (1–2 mg l⁻¹) and other body fluids. Although its function is still unknown, recent observations have markedly sharpened interest in this ubiquitous protein by suggesting an evolutionary link between β_2 -microglobulin, immunoglobulin (Ig) and the major histocompatibility (H) antigens.

The complete amino acid sequence of human β_2 -microglobulin has been determined and shows a striking degree of homology with the constant domains of human IgG (Cunningham *et al.*, *Biochemistry*, **12**, 4811; 1973). It has 100 amino acid residues, a molecular weight of about 11,500, a single intrachain disulphide bond which forms an intrachain loop of similar size to those seen in the homology regions of Ig, and contains no carbohydrate. These findings led to the suggestion that β_2 -microglobulin represented a free Ig domain with effector function similar to the terminal domain (C_H3) of IgG, which it resembles most closely (Peterson *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1697; 1972).

Two independent lines of evidence have indicated that β_2 -microglobulin is an integral part of the subunit structure of all the membrane proteins that carry the serologically defined major histocompatibility antigens (H-2 in mouse, HL-A in man) on the surface of virtually all normal nucleated cells. When HL-A (Cresswell *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603; 1973; Tanigaki *et al.*, *Immunology*, **26**, 155; 1974) and H-2 (Rask *et al.*, *Nature*, **249**, 833; 1974) antigens are solubilised by detergent or proteolytic enzyme treatment of cells, they are found to be composed of a heavy chain (~34,000 or 44,000 daltons depending on whether papain or detergent is used respectively)

and a light chain (~12,000 daltons) which appears to be identical with β_2 -microglobulin. There is evidence that both HL-A (Strominger *et al.*, *Transplantn. Rev.*, **21**, 126, 1974) and H-2 (Rask *et al.*, *Transplantn. Rev.*, **21**, 85, 1974) are four-chain structures in the membrane, with two heavy chains covalently linked by disulphide bonding between their hydrophobic tails, and the two β_2 -microglobulin molecules non-covalently bound to the heavy



Rask's tentative picture of an HL-A molecule in the cell membrane (from *Transplantn. Rev.*, **21**, 102; 1974).

chains, a structure remarkably similar to Ig. Characterisation of solubilised H antigens in guinea pig (Finkelman *et al.*, *J. exp. Med.*, **141**, 27; 1975), rat and rhesus monkey (Tanigaki and Pressman, *Transplantn. Rev.*, **21**, 15; 1974) have suggested that the β_2 -microglobulin-H association is a basic feature of H antigenic molecules in mammals.

A different approach to determining associations on the cell surface involves the binding of multivalent ligands (such as antibodies) to specific determinants (antigens or receptors) on the surface of intact cells to induce their redistribution into 'patches' or 'caps', and then studying the distribution of other membrane determinants. In such co-capping experiments, most antigens and receptors that have been studied redistribute independently, an exception being that all the HL-A antigens on human lymphocytes (Poulik *et al.*, *Science*, **182**, 1352; 1973; Solheim and Thorsby, *Tissue Antigens*, **4**, 83, 1974; Ostberg *et al.*, *Nature*, **249**, 463, 1974; Neaupoort-Sautes *et al.*, *J. exp. Med.*, **139**, 957; 1974) and other cell types (Solheim, *Transplantn. Rev.*, **21**, 35, 1974) co-cap with β_2 -microglobulin when the cells are treated with anti-

β_2 -microglobulin antibodies. These experiments make it clear that the association of solubilised HL-A with β_2 -microglobulin is not an artifact of the solubilisation and/or separation procedures. Capping HL-A with anti-HL-A antibodies fails to cap all of the β_2 -microglobulin, suggesting that not all of the β_2 -microglobulin is associated with HL-A.

Although β_2 -microglobulin occurs free in the serum, its structure makes it unlikely that it can be bound in the membrane other than by interacting with other membrane proteins. In mice two other plasma membrane proteins recently have been found to be associated with β_2 -microglobulin, the thymus leukaemia (TL) antigens (Ostberg *et al.*, *Nature*, **253**, 735; 1975), which are restricted to thymus lymphocytes and lymphoma cells, and the teratocarcinoma alloantigen(s) (Artz and Vitetta, personal communication), which is restricted largely to early cleavage and morulae stages of mouse embryos, spermatozoa, and teratocarcinoma cells. It is interesting that both of these alloantigens are determined by genes that are closely linked to the H-2 complex.

On the other hand, not all alloantigens coded for by genes in the H-2 complex are associated with β_2 -microglobulin; the Ia (immune response associated) alloantigens, determined by genes in the IR region of the H-2 complex, seem not to be associated with β_2 -microglobulin. In those cases where membrane polypeptides are normally associated with β_2 -microglobulin, it is unlikely that β_2 -microglobulin is required for their insertion or maintenance in the membrane. This is suggested by the finding that a human lymphoblastoid cell line from a patient with Burkitt's lymphoma (Daudi) does not synthesise β_2 -microglobulin or have it on its surface and yet probably carries the heavy HL-A polypeptide chain on its plasma membrane (Rask *et al.*, *Transplantn. Rev.*, **21**, 85; 1974). But it is possible that the β_2 -microglobulin serves to stabilise the functional conformation of these polypeptides (whatever their function turns out to be).

The structural linkage of β_2 -micro-

globulin with a variety of membrane proteins controlled by genes in or near the major histocompatibility complex raises the question of whether the gene for β_2 -microglobulin is also located in this chromosomal region. The report by Goodfellow *et al.* (*Nature*, **253**, 267; 1975) indicates that in man at least it is not. By studying a variety of human-mouse somatic cell hybrids they found that cells carrying human chromosome 15 expressed human β_2 -microglobulin on their surface, while cells not containing this chromosome did not; in a line which carried chromosome 15 as the only human chromosome, human β_2 -microglobulin was expressed. Since the HL-A complex has been assigned previously to chromosome 6, it seems virtually certain that in man, the β_2 -microglobulin and HL-A genes are unlinked.

Despite the apparent lack of genetic linkage, the physical association of β_2 -microglobulin with H or H-linked, membrane-bound gene products is intriguing. Taken together with the striking homology in structure between β_2 -microglobulin and some Ig domains, it suggests the possibility that β_2 -microglobulin, Ig and possibly these histocompatibility-linked genes have evolved from a common ancestral gene. The association of unlinked but evolutionarily related gene products to form a membrane subunit protein has a well established precedent in membrane bound Ig, where unlinked light (L) and heavy (H) chain gene products make up the subunit molecule H_2L_2 . Since membrane-bound Ig is known to function as an antigen-specific receptor on B lymphocytes, it may be that these other membrane molecules also have some kind of recognition or receptor role.

In the case of Ig both L and H chains have separately coded constant (C) and variable (V) regions, with V_L and V_H both contributing to the antigen-combining site; thus the multichain structure serves the obvious purpose of greatly increasing the potential number of combining sites that V_L and V_H gene products can form. Since there is no evidence for variability in the structure of β_2 -microglobulin, the grand purpose of the H- β_2 -microglobulin multichain structure remains a mystery. Unfortunately, very little is known about the structure of the histocompatibility heavy chains, and the possibility of their having separate constant and variable regions, or regions homologous to Ig domains $\pm$ β_2 -microglobulin, remains open.

The gene products of the H complex are mainly cell surface proteins, many of which are involved in various immune responses, particularly T lymphocyte responses, such as graft rejection, graft-versus-host responses, mixed lym-

phocyte reactions, cell mediated lymphocytotoxicity and other specific immune responses to a variety of antigens. Although the nature of the antigen-specific T cell receptor(s) remains controversial, and although it is not known how these H-complex gene products function, the physical association of β_2 -microglobulin with some of these surface proteins provides a suggestive evolutionary link between B cell-mediated humoral immunity and T cell-determined cell-mediated immunity. The possible immunological importance of some of the lymphocyte membrane proteins associated with β_2 -microglobulin is underlined by the recent findings that anti- β_2 -microglobulin antibodies inhibit various T cell responses (Solheim, *Transplant. Rev.* **21**, 35, 1974) and are mitogenic for B cells (Maller and Persson, *Scand. J. Immun.*, **3**, 100; 1974).

It is not surprising that immunologists are excited about β_2 -microglobulin.

Element 106 and onwards?

from C. J. Batty

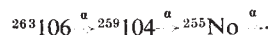
THE recent announcements by both Soviet and American teams of the discovery of element 106 is not only a further chapter in the saga of rival claims to be first but raises the interesting question as to how many other elements remain to be discovered, and in particular, what are the chances of producing superheavy elements.

To some extent the rival claims are due to the differences in techniques used by the Soviet and American workers. G. N. Flerov and his co-workers at the Joint Institute for Nuclear Research at Dubna near Moscow have generally used nuclear reactions involving fairly heavy ions and have looked for spontaneous fission activity. On the other hand A. Ghiorso and the team at the University of California, Berkeley, San Francisco have used heavier targets such as Californium, lighter incident ions and have made very detailed studies of the resulting α -activity. In both cases the techniques are difficult and the resulting activities short lived, with typical half lives of a few seconds or less. As a sign of the rival claims elements 104 and 105 have been christened Rutherfordium and Hahnium in the USA and Kurchatovium and Nielsbohrium in the USSR. Perhaps in an atmosphere of detente, element 106 so far remains unnamed.

Details of the Soviet work so far seem to be only available in unpublished reports but it involves the use of a radically different reaction method. Rather than using the heaviest target material available they instead chose lead. The nucleus ^{208}Pb has the advantage that it has 'magic' numbers

of both neutrons and protons and as a result the compound nucleus formed may have a low excitation energy. This leads to a decrease in the number of neutrons emitted and an increase in the cross section for forming the residual nucleus in its ground state. The use of lead rather than fissile material also helps in reducing unwanted background activities. The technique has been very successfully used by the Dubna group (*Nuclear Physics*, **A239**, 157; 1975) in producing two new isotopes of element 104 using the $^{208}\text{Pb}(^{50}\text{Ti}, 2n)^{256}\text{104}$ and $^{207}\text{Pb}(^{50}\text{Ti}, 2n)^{255}\text{104}$ reactions. In the work on element 106 they used a beam of ^{54}Cr ions and the team believe they have seen the $^{208}\text{Pb}(^{54}\text{Cr}, 3n)^{259}\text{106}$ reaction with the final nucleus decaying by spontaneous fission with a half life of between 4 and 10 ms^{-1} .

The work at Berkeley (*Phys. Rev. Lett.*, **33**, 1490; 1974) used a ^{249}Cf target, as in the previous experiments on elements 104 and 105, together with a beam of ^{18}O ions from the 'SuperHILAC' accelerator to study the $^{249}\text{Cf}(^{18}\text{O}, 4n)^{263}\text{106}$ reaction. Using a sophisticated system of α -detectors linked to an on-line computer they not only observed the α -decay of $^{263}\text{106}$ with an energy of 9.06 MeV and half life of $0.9 \pm 0.2\text{ s}$ but also observed the α decay of the daughter nucleus $^{259}\text{104}$ ($t_{1/2} = 3\text{ s}$, $E_\alpha = 8.8\text{ MeV}$) and of the grand-daughter ^{255}No ($t_{1/2} = 3\text{ min}$, $E_\alpha = 8.1\text{ MeV}$) so establishing the decay sequence



With very small cross sections for forming these very heavy nuclei together with their short half lives for decay it is clearly going to be difficult to go to the even heavier elements. But it is understood that the Dubna team are now trying to produce other isotopes of element 106 using their new technique and may attempt to synthesise elements 108 and 110 using beams of ^{58}Fe and ^{64}Ni ions. No doubt the Berkeley team have their own plans for work in this tough but exciting field.

Which leaves us with the question of the superheavy elements. These are an island of elements with atomic numbers in the region of 114 which owe their possible existence to the extra binding energy associated with the closed nucleon shells at 114 protons and 184 neutrons. Prediction of their half lives for decay is difficult but some theories have suggested values sufficiently long to warrant searching for them in natural materials on Earth (*Nature phys. Sci.*, **246**, 26; 1973) so far generally without success.

One problem with trying to produce these superheavy nuclei using the techniques employed for the 104 to 106 region is their very large neutron excess so that for example $^{298}\text{114}$ cannot be obtained by the complete fusion of any real combination of target and projectile. Nevertheless the Dubna group have reported (*Sov. J. nucl. Phys.* **19**, 123; 1974; *Sov. J. nucl.*

Phys. **19**, 247; 1974) experiments using beams of Zn and Ge ions, the latter being accelerated by a tandem arrangement of two cyclotrons. Looking for spontaneous fission activity with an experimental arrangement which permitted high sensitivity, the teams were unable to find any evidence for the production of super-heavies. It seems that further work will require even more sensitive experiments and higher intensity beams of energetic heavy ions.

Solar gravitational deflection of radio waves

from J. M. Riley

SINCE the publication of Einstein's general theory of relativity in 1916, there have been many attempts to test the prediction of this theory that electromagnetic radiation is deflected in the gravitational field of the Sun. The experimental test of the prediction involves measuring the change in the apparent position of a star when it is close to the Sun. The effect is extremely small as the predicted deflection amounts to a maximum of 1.75 arc s, for a ray path from a star which grazes the limb of the Sun, and decreases inversely with distance from the centre of the Sun. A very accurate measurement of this deflection is required as it is clearly of fundamental importance to find out how closely general relativity agrees with observation and to test its predictions against those of other theories of gravitation. Among the alternative theories which have received serious consideration is the Brans-Dicke scalar-tensor theory which, in its latest form, predicts that the deflection is 0.94 times that predicted by general relativity.

Until ten years ago the only tests had been carried out optically during total solar eclipses and, owing to great technical difficulties, had provided only semi-quantitative support for general relativity with accuracies of about 25%. Recently however it has become possible to perform the experiment at radio wavelengths using the technique of interferometry, in which the position of a radio source may be found by measuring the difference between the time of arrival of signals from the source at two radio antennae some distance apart on the Earth's surface; this method involves knowing with great precision the time at which the signals are recorded at each antenna. An experiment of this type has been performed several times since 1969 using the QSO 3C279 which passes behind the Sun in October every year;

it may be observed for several days before and after this when it is very close to the Sun. The change in its apparent position is measured relative to that of another source, 3C273, which is close to it in the sky but sufficiently far from the Sun during the experiment to be unaffected by the gravitational deflection.

The first tests of this kind were made using antennae separated by relatively short distances (from 1 to 5 km) operating at frequencies between 2,700 and 8,000 MHz; it was therefore possible to have direct electrical connection between the antennae and consequently it was relatively easy to standardise the time of the observations at the two antennae. The most accurate experiment of this type gave a result 0.96 ± 0.05 times the value predicted by general relativity.

It is also possible to carry out the experiment using antennae several hundreds of kilometres apart. In such an experiment the difference between the time of arrival of signals at the two antennae is proportionately greater than in the shorter baseline experiments and in principle therefore the experiment should be much more accurate. But in a long baseline experiment, direct electrical connection between the antennae is impossible so that independent clocks must be used at the two sites; due to instabilities in these clocks there is then a problem standardising the time of the observations at the two antennae. Furthermore, as the two sites are so far apart there are likely to be very significant differences in the path lengths through the atmosphere of the radiation arriving at the two antennae.

In a recent paper in *Physical Review Letters* (**33**, 1621; 1974) Counselman *et al.* describe the first results of such an experiment performed in October 1972 using a baseline of 845 km and a frequency of 8,000 MHz. In this experiment the problem of the differences between the independent standards used at the two sites was overcome by using a pair of antennae at each site, one directed at 3C279 and the other at 3C273. A single clock governed the recording of the signals received by both antennae at a given site; the difference between the time of arrival of the signals from 3C279 at the two sites could then be measured with reference to that between the time of arrival of the signals from 3C273 at the sites, thereby eliminating the effects of any clock instabilities at either place. This method also reduces the effects of any differences in the atmosphere and ionosphere above the two sites. In analysing the data it was necessary to assume models for the atmosphere, ionosphere and the solar corona, and uncertainties in these models, particu-

larly in that for the corona, contribute about half the error in the final result. The remaining error is attributed to experimental difficulties, in particular those introduced by fluctuations in the solar corona. The observed deflection was 0.99 ± 0.03 times the value predicted by general relativity. This measurement is probably the most accurate yet made of the gravitational deflection of radiation and the result is in close agreement with general relativity; it is slightly more than one standard deviation from the value predicted by the Brans-Dicke Theory.

The prospects for improving the accuracy of this test of general relativity using the method of very long baseline interferometry are good, as it is possible to eliminate several of the problems encountered in the experiment mentioned above by making simultaneous observations at two frequencies. It is likely that accuracies better than 1% may be achieved in the very near future.



A hundred years ago

WHITE'S "SELBORNE"

White's Natural History of Selborne.
Edited by J. E. Harting, F.L.S.
Illustrated by Bewick. (London:
Bickers and Co., 1875.)

ALTHOUGH we have no evidence that, within the last century, there has been any considerable change in the average standard of human mental power amongst civilised nations, the surroundings of every-day life have so greatly altered, both in their quality and in the rapidity of their occurrence, that the standard of ordinary existence has undergone a corresponding modification. The introduction of steam locomotion, the electric telegraph, and the penny post have developed such a condition of unrest in humanity at large that the unalloyed repose of a continuous rural life is rarely sought for, and as infrequently obtainable. We can hardly conceive it possible that anyone, such as a life-fellow of a college, as was Gilbert White, of Oriel, Oxford, should at the present day settle down in any out-of-the-way part of the country, satisfied with nothing more than an opportunity of observing and recording the surrounding phenomena of nature. More would be expected of him, and he would be continually led to feel that he was but one of the instances of the vegetating influence of an antiquated system, whose advantages were being daily disproved by his individual existence.

from *Nature*, **11**, 423; April 1, 1875

Causes of climatic change

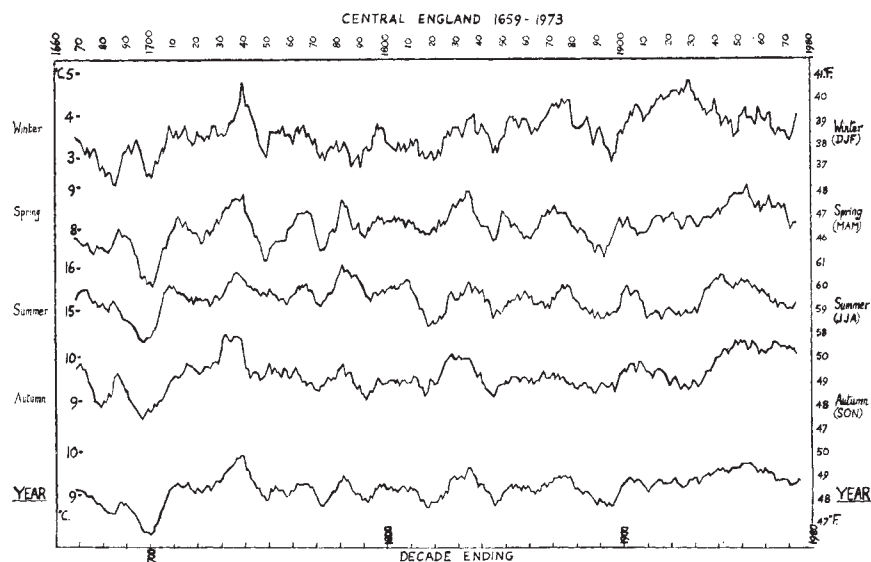
M. K. Miles of the Meteorological Office reviews the recent evidence while (below) John Gribbin reports on a lecture which may have helped to draw fresh ideas to the field.

FROM 1880, when the first reasonably reliable determination of the average temperature of the Northern Hemisphere was possible, there has been a warming (with only minor reverses) until 1940 and since then a cooling. In both cases the effect has been most marked north of about 70°N latitude and in the winter half of the year. From the 300-year-long temperature series prepared by Manley (*Q. J. R. Met. Soc.*, **100**, 389–405; 1974) for central England it could be said that winter temperatures have been rising since the end of the seventeenth century, at a rate of 0.36 °C per century with reverses lasting several decades. This temperature series indeed shows fluctuations on all time scales but there seem to be no clearly marked periodicities (see figure).

Possible effects of sunspots

Many investigators have sought a relationship with the various periodicities displayed by sunspot activity, and although for some records and some seasons statistically significant results have been obtained, there are also contradictory results. If there is a real relation with the 11-year sunspot cycle it is a complicated one, changing its phase relationship from epoch to epoch, but in any case not accounting for a very substantial amount of the variance at that time scale. The same applies to the double sunspot cycle (successive 11-year cycles showing opposite magnetic polarity of the sunspots).

A relation with a longer cycle of solar spot activity varying between 60 and 100 years has been claimed, but the amplitude of the response is small and not clearly above the play of chance. There is naturally even less convincing evidence for a suggested periodicity of 180–200 years related to a solar cycle whose length and physical characteristics are not well established. The ¹⁸O record from the Camp Century ice core has been adduced as evidence for periods of 77 and 177 years, but adjustments were made to the time scale to preserve persistent climatic oscillations so it is not entirely independent evidence. Even so the record is substantially out of step with the



Decadal running averages of seasonal and annual mean temperatures, from Manley, *Q.J.R. Met. Soc.*, **100**, 402; 1974.

Manley temperature record for central England in the eighteenth and nineteenth centuries. The variations of ¹⁴C content in dated wood specimens have been claimed to show 200- and 400-year periodicities, but the experimental error in the ¹⁴C determinations means that such a claim should be accepted with great reserve.

It is still not certain that the heat output from the Sun varies enough with changes in the number of sunspots to affect the lower troposphere. Satellite measurements indicate large changes in the ultraviolet part of the spectrum at different times during the 11-year sunspot cycle, but the fraction of the total energy in this part of the spectrum is very low. To date satellites have not monitored changes in the spectral range 0.3 to 1.0 μm.

Terrestrial influences

Other factors thought capable of producing changes in the lower troposphere are dust (volcanic and wind erosion dust), CO₂, water vapour and changes in ocean temperature and salinity. Increasing CO₂ from burning of fossil fuel is generally estimated to be capable of explaining a substantial part of the warming since 1880, and a clearer stratosphere resulting from fewer volcanic eruptions in the twentieth century compared with the eighteenth and nineteenth would be expected to act in the same sense. If this be so then a strong cooling factor must have come into play since 1940. The first major eruptions after Katmai in 1912 did not occur until the early 1950s, so a sudden increase in dust from other sources (and aerosol loading) has been postulated. Measurements of the electrical conductivity of the air over

the Atlantic indicate an increase in the number of Aitken nuclei in recent years compared with the early 1930s but the data are not adequate to show whether this happened around 1940 or not. On the other hand direct solar radiation measurements as collated by Budyko and Vinnikov (*Glav. Uprav. Gidromet. Sluz. Met. Gidr.*, **9**, 3–13; 1973) do not reveal a substantial drop until about 1945. The dust from semi-arid zones in America and the Soviet Union which one would expect to have increased following the dry conditions of the 1930s does not seem to have affected radiation.

Aerosols from increasing industrial activity and changing agricultural practices are probably the most likely agents to have contributed to the fall in direct solar radiation said to have occurred since 1945. There are, however, two factors to be considered before it may be concluded that the cooling is due to this. First, the aerosol being largely in the troposphere will often lie below cloud top, and does not have a long residence time in the atmosphere. Second, it may absorb radiation as well as reflecting it so that the net cooling effect may be very small. However, it must be said that attempts to determine the secular change of atmospheric turbidity do not all support an increasing global trend either for the time since the late 1950s or for the century.

Quite small variations in the global cloud cover are capable of altering the heat balance substantially but data are not adequate to reveal any long term trend. Similarly data about ocean temperature and salinity are not adequate to assess the part the oceans might have played in the warming and subsequent cooling. Minor changes in

oceanic circulation are capable of leading to substantial changes in the heat exchange with the atmosphere.

The area of ice and snow cover in the polar regions decreased between 1900 and 1930, then began to increase in the late 1950s. One school of thought attributes the increase to the lower direct heat input since about 1945, but the decline in the Atlantic winter circulation in the last two to three decades could perhaps have produced the same effect. There is even some evidence that a further change occurred about 1970. The area of sea ice was less for the four years 1971–74 than for the preceding four years. Again there has been a change in the Atlantic winter circulation but it is noteworthy that Budyko in an updating in 1973 of his direct radiation index shows an increase setting in during the late 1960s.

Other atmospheric variation

Changes in precipitation have also received attention—local changes as well as regional distribution—and various claims have been made for periodic fluctuations. The supporting evidence has not however been strong enough to lead to a consensus of

opinion. For example the 22 years of the double sunspot cycle is claimed (and widely accepted) to be related to the incidence of droughts over the plains of the American Middle West but data for the steppe and forested zones of the Soviet Union do not show the same pattern.

Fluctuations in the strength of the general wind circulation in the Northern Hemisphere broadly parallel the temperature behaviour though there is an indication of a lead of about 10 years—the peak in the hemispheric westerlies for the latitude band 35–55°N being reached by 1930. The North Atlantic winter circulation which displays these changes to a marked degree certainly declined after 1930 whatever latitude band is examined. It is difficult to relate these or the smaller scale variations in a sensible way with changes in the temperature gradient between the tropics and the pole or suspected changes of the meridional heat flux. Only a small fraction of the internal energy of the atmosphere is converted to kinetic energy and so there is no reason to expect a simple relation between energy input and circulation energy.

The reality of strictly periodic fluc-

tuations in the Earth's climate being to some extent in doubt and trends in various parameters being either doubtful (as for example turbidity) or not yet distinguishable from random fluctuations, satisfactory predictions for the next few decades cannot be made. Perhaps all that is justified are estimates of the range of possible future fluctuations based on past climatic data together with assessments of the influence of human activity on the surface of the Earth and the composition of its atmosphere.

Protecting damaged plant life of Galapagos

from Peter D. Moore

THE impact of man and his domesticated animals upon the unique flora and fauna of the Galapagos Islands has been the source of considerable concern in recent years. Schofield (*Biol. Cons.*, 5, 48; 1973) considered that many endemic trees and shrubs, such as *Scalesia pedunculata* and *Miconia robinsoniana*, were threatened both as a

ON March 10, Dr Peter Wright (University of East Anglia) spoke at the University of Bristol about climatic change to an audience of mixed physical scientists. He emphasised that climate is always changing, on many timescales, and that these changes occur globally. A disturbance of climate in one part of the world will affect the whole system, with possibly drastic repercussions on the other side of the globe. But man's direct influence on climate, through heating of cities, for example, seems insufficient to disturb the global machinery. The kind of disturbance cited by Wright as important would be about 1,000 km across and persistent—paving over the interior of Brazil, say; or smaller and more energetic, but persistent—exploding a nuclear device in the same spot every day for ten years. So it is still the study of 'natural' climatic fluctuations which is of key importance.

Wright dismissed most of the proposed natural causes of change as minor contributors. Solar variations (over the sunspot or longer cycles) could play a part, but are unlikely to dominate to the extent of causing ice ages; the Milankovitch theory that changes in the Earth's orbit and inclination affect insolation enough to cause ice ages seems more tenable (especially now that the geological record has been re-

New climatologists begin here

interpreted to give a better guide to the variations of glaciation over the past few million years); dust in the atmosphere from volcanoes or blown away from eroded and desert areas is a good candidate for irregular effects, but is difficult to reconcile with cyclic changes in climate (if, that is, you believe that climatic changes are cyclic, which Wright does not); geomagnetic fluctuations could, says Wright, 'just conceivably' play a small part (others might argue that by varying the flux of cosmic rays reaching the atmosphere such changes in magnetism could be of greater importance); and man's influences have certainly not been important as yet.

All of these processes are forcing effects; Wright, however, believes that, although such variations may make conditions more suitable for, say, icier conditions, it is feedback that maintains any climatic regime.

For example if a patch of ocean in the northern hemisphere becomes warmer than average, the effect will tend to be to shift the circum-hemispherical wind streams so that instead of blowing from west to east across the warm patch they swing

up from the south-west, pass around the north of the warm patch and swing away to the south-east. The effect is to bring warmer air over the 'anomaly', tending to maintain its warmer state.

A more realistic example of a feedback system is the "Southern Oscillation", in which a patch of ocean along the equator just west of the Americas fluctuates between a colder and less cold state (the difference is no more than 3 °C) with associated changes in the pattern of trade winds. Each state seems stable, and each is maintained by feedback between wind and ocean systems. Why should the situation ever change? Clearly there are external factors involved, even in the rest of the air-ocean system, and computer modelling is far from the stage of sophistication where all these effects are included.

As Wright pointed out, climatology today is still to a great extent in the data gathering stage; but already many data are available for analysis and more physical ideas are needed for testing both in the real world and in the models—and this is where there seems to be scope for new talent. Outsiders with experience in fluid mechanics, thermodynamics, statistical interpretation of noisy data and so on could play a vital part in the interpretation of the data as they are collected.

result of intensive clearance and cultivation of upland areas and because of the spread of exotic plant species introduced by man (see *Nature*, **242**, 299; 1973). Davies (*Nature*, **249**, 788; 1974) pointed out the threat to the islands' flora presented by feral goats and the problems in carrying out scientific research and conservation on the inadequate budget of the Charles Darwin Research Station. Can one be sure that the demise of the Galapagos flora and fauna will even be recorded adequately?

A research programme to monitor habitat changes in the islands was instigated by the research station in 1966. Permanent plots of between 6.25 m² and 100 m² were established on several islands in habitats which were thought to be threatened, and recordings have been made since that time of vegetational changes within the plots. Hamann (*Biol. Cons.*, **7**, 37; 1975) has now presented some of the data emerging from this study over the period 1966–73.

The arid zone vegetation has suffered to different degrees on the various islands. On Pinta, where goats have in the past been a most serious pest, the denudation of vegetation has been severe, sometimes resulting in soil erosion. Recent reduction in goat population density has not yet resulted in any marked recovery of the vegetation. There is however cause for optimism in view of the regeneration which has been noted on Santa Fé, where feral goats were exterminated in 1971. *Cordia lutea*, *Encelia hispida*, *Lantana peduncularis* and *Scalesia helleri* are all recovering in the arid zone of this island since the cessation of grazing. One might hope that regeneration will occur in Pinta also, if goat populations can be further reduced.

The *Scalesia pedunculata* forests of Isla Santa Cruz, although badly affected by grazing and clearance, seem to have the capacity for rapid recovery and growth under suitable conditions. Recent intensive hunting of introduced mammals by National Park wardens may be responsible for the observed increase in saplings of *Scalesia* within the plots. However, cattle seem to be very partial to young *Scalesia* trees, so full recovery can only occur if cattle grazing is controlled. Exclosure plots could supply the answer to this.

Perhaps the most sensitive vegetation type of the Galapagos is that dominated by *Miconia robinsoniana* with *Cyathea weatherbyana*. Much of this zone has been damaged by burning, followed by cattle grazing, and this has resulted in its replacement by ferns, particularly *Pteridium aquilinum*. It may be that recent prolonged droughts have also contributed to the failure of

regeneration in *Miconia*. This vegetation type will undoubtedly provide the most difficult conservation problem of all on the Galapagos. It has almost disappeared from San Cristóbal and is failing to recover on Santa Cruz, its only other station. Protection of the zone from further burning and grazing must be regarded as a management priority.

The control of introduced mammals on the Galapagos has proved possible and worthwhile. It is to be hoped that funds will be found to continue and intensify this programme of conservation and that schemes can be initiated which will satisfy the needs of both farmer and conservationist on the islands.

Long period of lunar crater formation

from Peter J. Smith

ONE of the hopes not entirely fulfilled by the Apollo missions was that it would be possible to recover a significant number of lunar rocks older than 4,000 million years. As terrestrial rocks of corresponding age have never been found, some workers had hoped that access to lunar material would enable important light to be thrown on the first 500–600 million years' evolution not only of the Moon itself but also of the Solar System and particularly the Earth. Unfortunately, most lunar mare and highland basalts dated radiometrically appear to be between 3,950 and 3,130 million years old. It is true that some highland rocks, notably the cataclastic anorthosites, have yielded ages much closer to that of the Solar System; but some doubt has been expressed as to what such great rock ages really mean. The difficulty is that much of the lunar regolith in the appropriate regions represents ejecta from the major basins, material which has been severely shocked; and it is not entirely clear just how shock affects a rock's radioactive properties.

Recently, however, Gooley *et al.* (*Geochim. Cosmochim. Acta*, **38**, 1329; 1974) described an unshocked troctolite (Apollo 17) comprising plagioclase, olivine and bronzite, which they conclude cooled at a rate of a few tens of degrees per million years at a depth of 10–30 km. Two whole rock samples and a plagioclase separate from this material have now been dated by Husain and Schaeffer (*Geophys. Res. Lett.*, **2**, 29; 1975) using the ⁴⁰Ar–³⁹Ar technique. Extensive tests and close agreement between the ages obtained from all three samples convince Husain and Schaeffer that the accurate mean

age of the troctolite is 4,260 ± 20 million years.

The corresponding ³⁸Ar/Ca cosmic ray exposure age is 156 ± 8 Myr, suggesting that the troctolite was last brought to the surface comparatively recently. The much older ⁴⁰Ar–³⁹Ar age, on the other hand, supports the major conclusion from Gooley and his colleagues that the rock was originally a product of large scale igneous processes which formed the early lunar crust. The combined evidence from petrography and the Ar systematics is that the troctolite probably formed about 4,500 million years ago and then cooled very slowly at depth for tens to hundreds of millions of years. During most of this phase the temperature was apparently sufficiently high to make the rock an open Ar system. But for the past 4,260 Myr the rock has evidently been a closed Ar system, thus enabling one to date the time at which it was excavated from depth and reburied under an ejecta blanket.

This is the simplest and most reasonable chronology consistent with the available data. But Husain and Schaeffer believe that they can go even further in relating the rock's history to named events on the Moon. For (shocked) lunar breccias from Apollos 14–17, the histogram of measured ⁴⁰Ar–³⁹Ar ages (see, for example, Schaeffer and Husain, *Geochim. Cosmochim. Acta*, Suppl. **4**, 1847; 1973) covers the range 3,900–4,260 million years and is sharply peaked at 3,900–4,000 Myr. As the breccias were almost certainly formed by impacts on the lunar surface and as extensive Imbrium ejecta exist at the Apollo 14–17 landing sites, the histogram peak strongly supports the view that the Imbrium event occurred between 3,900 and 4,000 million years ago.

If this is so, the older breccias (assuming their measured ages to be reliable) and the Apollo 17 troctolite must be related to earlier events. As far as the troctolite is concerned, the impact which excavated it is now identified by Husain and Schaeffer as the Serenitatis event, largely because the Apollo 17 site lies at the rim of the Serenitatis basin. If this is correct, it follows that the age of the Serenitatis is probably 4,260 Myr, the age of the troctolite. This, in turn, suggests that what Husain and Schaeffer term the "lunar basin forming era" lasted at least 300 Myr (Serenitatis–Imbrium). In fact it may have lasted much longer because the Serenitatis impact event was preceded by the Fecunditatis, Tranquillitatis and Nubium events. In any case it now appears that these major lunar basins could not all have been formed in a relatively short time about 4,000 million years ago, as some workers have suggested.

review article

Schlieren experiment 300 years ago

J. Rienitz*

Schlieren techniques for making visible density gradients in transparent media seem to be older than had been supposed. Robert Hooke developed a method of this kind as early as 1672 but, unlike Christian Huygens, who described a similar technique in 1685, obviously aroused no interest.

DURING the long decades of his activity as Curator of Experiments of the Royal Society of London, Dr Robert Hooke (1635–1703) developed a large number of ideas, experiments, and instruments in the fields of science, technology, and medicine^{1,2}. Lack of time to elaborate many of his ideas, undeserved discredit, the change in the conception of scholarship during the Newtonian Age, and rivalries in the edition of his bequeathed papers have meant that much of his work has remained unknown. One of his unrecognised achievements seems to have been the development in 1672 of a true schlieren method. Commonly, it is Léon Foucault (1859) and August Toepler (1864) who are regarded as the discoverers of this group of techniques^{3,4}.

After the invention of the microscope and the telescope at the beginning of the seventeenth century activity in the field of optics became widespread. Refraction was investigated thoroughly, methods for improving the lenses as well as the performance of the optical instruments in general were sought, and the reflections of scholars on the nature of light began to gain impetus. Hooke's interest in astronomical problems, as well as in the mechanics of gases, led him to investigate atmospheric refraction. He treated it extensively in Observation LVIII of his famous *Micrographia* of 1665 together with a number of related phenomena, writing, for example, "This I have likewise often observ'd in a hot Sunshiny Summer's day, that looking on an Object over a hot stone, or dry hot earth, I have found the Object to be undulated or shaken, . . .". His explanation is quite correct: "a multiply refracted, caused by the unequal density of the constituent parts of the medium, whereby the motion, action or progress of the Ray of light is hindered from proceeding in a straight line, and inflected or deflected by a curve"⁵.

These density gradients in a transparent medium are termed streaks. Hooke mentions streaks in gases and vapours, in fluids, and in solids. The unequal refraction which is the consequence of the unequal optical density is barely visible to the naked eye, and hence the development of schlieren techniques which enhance the contrast. Robert Hooke mentioned his schlieren set-up first when he was treating the combustion of a candle as a part of his extensive but often overlooked^{1,6} investigations on the nature of combustion, in the course of which, incidentally, he clearly realised the function of oxygen¹. The experiment was performed by Hooke before the Royal Society on February 22, 1671 old style (or March 4, 1672, Gregorian calendar) "to shew, that, besides the flame and smoke of a

candle there is a continual stream rising up from it, distinct from the air"⁷. A week later, the experiment "was repeated and proved satisfactory"⁷. Finally, on March 14, Hooke submitted an account of the experiment which was registered⁷. Here, he describes first the method. (For sake of clarity the notations of the illustration are inserted in the following original text in square brackets.)

"I took a large concave reflecting glass, or a large convex refracting glass [L], and so placed in respect to my eye [E], that a candle [C₁] set at a certain distance beyond the refracting glass, or between the eye and the superficies of the reflecting glass, enlightened the whole area of the said glasses in respect to the eye. Then continuing to keep the eye in that place, where the area of the said glasses appeared to be wholly filled with the flame of the candle, I caused another candle [C₂] to be placed very near the said glasses, between the eye and the glass; or beyond also, if I made use of the refracting glass [L]. Then looking stedfastly at the flame of this last candle, it was very plain to be perceived, that the flame thereof was encompassed with a stream of liquor, which seemed to issue out of the wick, and to ascend up in a continual current, or *jet d'eau*, to keep itself intire, and unmixed with the antient air, notwithstanding that it was a considerable way carried above the aforesaid flame"⁷. Then he mentions "several turnings, whirlings, or vortices in the ambient air" familiar to all who ever have observed a burning candle in a schlieren apparatus. In Fig. 1, S may designate a region of streaks in homogeneous surroundings. Owing to the gradient of refraction the bundle of rays R penetrating this inhomogeneity will be deflected more or less from the pupil of the eye E so that the streaks stand out more or less dark against the bright background. The pupil of the eye plays here, in principle, the same role as the knife edge in the methods of Foucault and Toepler^{4,8}.

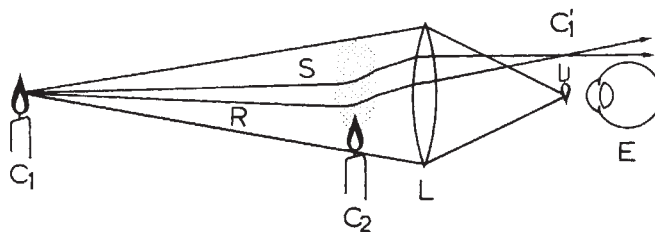


Fig. 1 A reconstruction of Robert Hooke's schlieren set-up. C₁ the first (illuminating) candle; L the lens; E the eye of the observer; C₁' the image of C₁ produced by L, in reality at the pupil of E; C₂ the second candle; S a region of streaks above C₂; R a bundle of rays of light passing through S. For clarity the distances of C₁ and E from L are represented as smaller than they may actually have been.

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but at present schlieren systems with a circular cutoff instead of the knife edge are also used².

Hooke explained the operation of his method in almost the same way: "The manifestation of this phenomena [sic] was from differing refractions of the body of the jet d'eau from that of the ambient air: for the flame of the first candle being but small, and placed at a considerable distance from the refracting, or reflecting glass, the smallest variation in the refraction of the medium between the first glass and the eye caused the darkness to intermix with the light; so to exhibit the appearance of the heterogeneous jet d'eau"⁷.

These words show, too, that Hooke obviously realised at least one of the conditions mentioned by Mach⁸ for ensuring the sensitivity of the schlieren method: that is, a small light source. The subsequent passage which states that the first candle is "placed at a considerable distance from the refracting, or reflecting glass" is, however, ambiguous. It could simply refer to long focal distances, another condition of sensitivity mentioned by Mach⁸. On the other hand, it could refer to the first condition and signify that the flame of the first candle is imaged on the pupil of the eye at a reduced scale (Fig. 1). Since even a small candle flame has an area greater than that of the pupil, the area of its image may by these means be made comparable with that of the pupil, so that a minute difference of refraction at a certain region of the object causes at least partial non-coincidence of both areas and hence a darkening in the image of that region. This seems, however, to be valid only for the lens, for in the case of the mirror this interpretation contradicts the passage cited above which says that the first candle should be placed "between the eye and the superficies of the reflecting glass".

After having submitted his account, Hooke "promised to exhibit at the next meeting, an experiment to shew a phaenomenon not unlike this, to be produced by several bodies dissolved in oil of vitriol"⁹. On March 28 of the same year he performed an experiment of dissolving sal-nitre in common water. Here, a stream is observed "composed of water and of the particles of nitre dissolved therein" which "was here descending, as in the former experiment a stream or fluid produced by a candle dissolved by the air ascended"⁷. This seems to be the experiment promised by Hooke, but he may have been mistaken or changed his mind about the appropriate substances. Later on he demonstrated experiments on mingling oil of vitriol and water, but here no observations of streaks are reported.

Hooke mentioned his schlieren experiment once more in his Cutlerian Lecture entitled "Lampas", which was published in 1677, although so briefly that it is recognisable only by those familiar with it: "This Figure and shape of the flame and vapours may be plainly seen by the help of a Metalline Concave placed at a certain distance and Position, and also by observing the shadow of the candle cast by the beams of the Sun upon a sheet of white Paper, or white Wall, but the way of a Concave *speculum* is incomparably beyond it, because it doth so very plainly shew the form and manner of the streams rising above . . ."⁹. The second, less sensitive procedure mentioned here is the same in principle as the shadowgraph method introduced into schlieren optics by Dvorak in 1880^{3,10}. The same effect can be observed when the sun is shining through blown window panes.

Finally, Hooke repeated his experiment before the Royal Society on January 16, 1678 (or January 26, 1679, Gregorian calendar), and the short report ends: "This was plainly seen by the president and divers others of the members present to their satisfaction"⁷. At the same date Hooke records briefly in his diary: "Shewn experiment of . . . flame"¹⁶.

At much the same time another schlieren experiment had been performed by Christian Huygens¹¹. He illuminated the

back of a convex lens with the reflected light of a candle in a way similar to Hooke's method, observing the reflection in order to test the surface for defects of polish, as well as the glass for streaks. These defects of polish are local irregularities in the surface which, analogous to streaks, deflect the light into another direction than their surroundings and can therefore be made visible in the same way by schlieren methods. With lenses of great focal length Huygens even used a small telescope to observe the schlieren image, a fact Toepler emphasises as an advantage of his method compared with that of Foucault⁴.

Huygens certainly developed his method without knowledge of Hooke's procedure, for if he had been acquainted with it, he would not have hesitated to repeat the experiment with the candle which is still today one of the most spectacular demonstrations in schlieren optics. He seems to start from his thinking of the laws of image formation in terms of the "point de confusion maximum" (point of maximal blurring), by which he means simply the image point of an object. If one observes a candle through a lens, the image of the candle becomes increasingly blurred as the eye moves back from the lens. At the point mentioned above the blurring reaches a maximum because the whole aperture of the lens is filled with light and the image of the candle is lost. As the distance of the eye from the lens increases, the image becomes clearer once more and finally, one is able to discern an inverted image of the candle. Hooke's description of his method shows that he was familiar with this observation too.

This concept has been used by Huygens in his *La Dioptrique*¹² published in 1653, but his schlieren method was evidently invented much later. It was first published posthumously in 1703, but the editors of his *Oeuvres Complètes* fix it at 1685¹¹, 13 years after Hooke. This date seems to be justified, for in a letter to his brother Constantyn who had often made telescope lenses with him, dated October 7, 1686, Christian Huygens described his method as if it were new and added the words: "Je voudrais que vous fussiez icy pour estre present a cette epreuve"¹³ (I wished you were here in order to be present at that test).

Huygens' method, though rarely mentioned in literature, has obviously not been completely forgotten by the experts, as has been the fate of Hooke's findings. The well known glassmaker Pierre Louis Guinand, who collaborated later on with Fraunhofer, reported that he became acquainted with it in 1798 when he was visiting the opticians at Paris¹⁴. It seems very probable that the news of Huygens' method reached Paris through the well known work *A compleat System of Opticks* by Robert Smith¹⁵, first published in 1738. Huygens's treatise had originally been written in Dutch, but was later translated into Latin (by Hermann Boerhave, according to Abraham Gotthelf Kästner in the German translation of Smith's book). Smith certainly made Huygens's ideas better accessible by reporting them in English. Since, however, the very skilled optician Mitoufflet Thomin in Paris was obviously ignorant of these techniques in 1749¹⁶, one may assume that it was the publication of the French translation of Smith's work in 1767¹⁷ that changed the situation. A connection between the knowledgeability of the opticians at Paris and Léon Foucault may thus not be completely excluded.

The relatively detailed accounts of Hooke concerning streaks, as well as his schlieren set-up, are essential for interpreting the scanty and rare hints at microscopical methods related to schlieren techniques that have been dropped by Hooke and some other old microscopists. Such investigations are now in progress.

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⁴ Nebe, W., *Jenaer Jahrbuch*, 1964, 11-37 (Fischer, Jena, 1965).

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articles

Possible power source of Seyfert galaxies and QSOs

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The possible presence of massive black holes in the nuclei of galaxies has been suggested many times. In addition, there is considerable observational evidence for high stellar densities in these nuclei. I show that the tidal breakup of stars passing within the Roche limit of a black hole initiates a chain of events that may explain many of the observed principal characteristics of QSOs and the nuclei of Seyfert galaxies.

STARS of mean density ρ are broken apart by the tidal force of a black hole of mass M if they pass within a distance

$$R_T \simeq (6M/\pi\rho)^{1/3} \quad (1)$$

of it. They are physically absorbed by the black hole if they pass it within the gravitational radius,

$$R_G = 2GM/c^2 = 2.95 \text{ km } (M/M_\odot) \quad (2)$$

R_T and R_T/R_G as functions of M are given in Table 1. I have assumed solar type stars in evaluating ρ in equation (1). For $M < 3 \times 10^8 M_\odot$, the tidal radius is greater than the gravitational radius so that stars are far more likely to be broken apart by the black hole than absorbed whole. By the time the black hole has grown to the critical mass $M_c \simeq 3 \times 10^8 M_\odot$, it may have produced several times its own mass in gas, by breaking up stars. This may be the source of the net outflow of gas observed in the nuclei of many galaxies.

Capture of gas by the black hole

Much of the gas produced by tidal breakup is captured and subsequently accreted by the black hole. The tidal breakup of a star requires energy which is ultimately supplied by a reduction in the kinetic energy of the star with respect to the black hole. The energy required to break up a star of mass m and radius R is

$$E_b = \frac{3}{2}(Gm^2/R) \quad (3)$$

for a polytrope of index $n=3$. The gas produced by the breakup of a solar type star is captured into a bound orbit by the black hole if the velocity of the star with respect to the black hole

before their encounter was less than 535 km s^{-1} . Thus if the velocity dispersion of the stars in the nucleus of a galaxy is less than about 500 km s^{-1} , much of the gas produced by tidal breakup is captured and ultimately accreted by the black hole.

For the larger black holes, which have $R_T \sim R_G$, emission of gravitational radiation may also be important in reducing the kinetic energy of the star with respect to the black hole¹, but I shall not consider this additional loss mechanism here.

The gas produced by the breakup of a particular star may travel in a bound orbit to a large distance from the black hole, but eventually it must return to the original periastron distance of its stellar precursor. The mean semi-major of the gas produced by the breakup of a solar type star by a black hole of mass M is

$$r = (2M/3m)R[1 - 2\langle V^2 \rangle R/3Gm]^{-1} \\ = 1.5 \text{ pc } (M/10^8 M_\odot)[1 - \langle V^2 \rangle/(535 \text{ km s}^{-1})^2]^{-1} \quad (4)$$

where $\langle V^2 \rangle$ is the mean-squared velocity of the stars in the galactic nucleus before their encounter with the black hole. Thus a $10^8 M_\odot$ black hole is surrounded by a massive gas cloud with a typical radius of 10^{-2} pc . The dust in this cloud strongly absorbs the ultraviolet radiation emitted by the gas being accreted by the black hole. This may be responsible for the infrared emission of QSOs and Seyferts. The ionised gas in this large cloud may in turn be responsible for the forbidden-line radiation from QSOs.

Collisions among the gas clouds originating from the breakup of individual stars will release enormous amounts of energy. High velocity collisions between gas clouds can explain several phenomena associated with QSOs (ref. 2). The dissipation of energy from collisions reduces the effective semi-major axis of the gas until it is well within the Roche limit. Ultimately the gas must be accreted by the black hole unless it is stopped by rotation. Even if this occurs, the gyration radius of the gas must be much less than the Roche limit of the black hole unless the gas acquires additional angular momentum after the breakup of its stellar progenitors by interacting with the stars around the black hole. If the angular momentum is sufficiently large that the gas settles into a disk spinning around the black hole, viscous forces eventually will allow most of this gas to be accreted by the black hole as in an X-ray binary^{3,4}. The accretion of gas by a massive black hole produces a large amount of ultraviolet radiation⁴ which will tend to ionise the massive gas cloud around the black hole to produce the observed forbidden-line radiation and the cores of the permitted lines. The Doppler broadening

Table 1 Breakup and accretion of stars by a black hole

$M/M_\odot$	$R_T/R_\odot$	R_T/R_G	$L_E/L_\odot$ (Eddington Limit)	t_E (yr)	L/L_E ($\rho_s = 10^6 M_\odot \text{ pc}^{-3}$)	t_D (yr)
10^1	4.31	1.02×10^5	3.2×10^5	1.6×10^9	1.5×10^{-3}	1.8×10^{11}
10^2	9.28	2.19×10^4	3.2×10^6	1.4×10^9	3.2×10^{-3}	8.4×10^{10}
10^3	20.0	4.72×10^3	3.2×10^7	1.2×10^9	7.0×10^{-3}	3.9×10^{10}
10^4	43.1	1.02×10^3	3.2×10^8	9.4×10^8	1.5×10^{-2}	1.8×10^{10}
10^5	92.8	2.19×10^2	3.2×10^9	7.3×10^8	3.2×10^{-2}	7.9×10^9
10^6	200.0	4.72×10^1	3.2×10^{10}	5.2×10^8	7.0×10^{-2}	3.3×10^9
10^7	431.0	1.02×10^1	3.2×10^{11}	3.2×10^8	1.5×10^{-1}	1.2×10^9
10^8	928.0	2.19	3.2×10^{12}	1.1×10^8	3.2×10^{-1}	2.7×10^8
3.2×10^8	1,374.0	1.00	1.0×10^{13}	0	4.8×10^{-1}	0

resulting from the rotation of the gaseous disk around the black hole and the large random velocities of dense colliding gas clouds near the black hole may be responsible for the large widths of the permitted emission lines in Seyferts and QSOs.

Luminosity and total energy

It is evident that most of the mass acquired by a black hole less massive than $M = 3 \times 10^8 M_\odot$ is from the accretion of gas rather than of whole stars. In this case the total energy released by this accretion is $E = 0.057\text{--}0.43 Mc^2$ depending on the angular momentum of the black hole⁶. If $E = 0.2 Mc^2$, the total energy released by the black hole by the time its mass reaches the critical value $M_c = 3 \times 10^8 M_\odot$ is $E_c = 1.1 \times 10^{62}$ erg $\equiv 9 \times 10^{20} L_\odot \text{ yr}$. The maximum luminosity produced in the accretion of material by the black hole is given by the Eddington⁶ limit,

$$L_E = 3.2 \times 10^4 L_\odot (M/M_\odot) \quad (5)$$

Thus the maximum possible luminosity produced by the accretion of gas by the black hole is $L = 10^{13} L_\odot$ which occurs when $M = M_c$. This is very near the peak observed luminosity of QSOs. The total energy release could power a quasar at this luminosity for as long as $9 \times 10^7 \text{ yr}$.

If $E = 0.2 Mc^2$, the rate of mass accretion by a black hole growing at the Eddington limit is

$$dM/dt_E = 1.1 \times 10^{-8} M_\odot \text{ yr}^{-1} (M/M_\odot) \quad (6)$$

In this case, the time required for a black hole to grow from a mass M to $M_c = 3 \times 10^8 M_\odot$ is

$$t_E = 9.3 \times 10^7 \ln(M_c/M) \text{ yr} \quad (7)$$

Table 1 shows t_E and L_E as a function of M . I assume that the luminosity of a QSO drops by orders of magnitude when its mass reaches $M = M_c$ at which point the stars are swallowed whole by the black hole rather than being broken apart tidally first.

I note (Table 1) that a seed black hole growing at the Eddington rate takes about $1.6 \times 10^9 \text{ yr}$ to increase its mass from about $10 M_\odot$, a mass one may expect from stellar evolution, to $M_c = 3 \times 10^8 M_\odot$. So the observed number of QSOs in the Universe would decrease very rapidly after the Universe was more than $1.6 \times 10^9 \text{ yr}$ old. If the Universe is presently $1.2 \times 10^{10} \text{ yr}$ old, this critical time corresponds to $Z = 2.8$ in an Einstein-de Sitter universe. This is consistent with the observations of Schmidt⁷ that the density of QSOs in the Universe increases as $(1+Z)^6$ up to $Z \simeq 2.5$ and then it either remains constant or decreases for larger Z .

Growth governed by stellar density

If the density of stars in the nucleus of a galaxy is not sufficiently large, this density rather than the Eddington limit governs the rate of growth and luminosity of the black hole. Assuming

Newtonian mechanics, the rate at which gas is being produced by the breakup of stars passing within the Roche radius R_T of a black hole of mass M is

$$\begin{aligned} \frac{dM}{dt} &= \rho_s \langle \sigma V \rangle \\ &= 8.6 \times 10^{-16} \frac{M_\odot}{\text{yr}} \left[\frac{\rho_s}{M_\odot/\text{pc}^3} \right] \left[\frac{R_T}{R_\odot} \right] \left[\frac{M}{M_\odot} \right] \left[\frac{\text{km s}^{-1}}{\langle V^2 \rangle^{1/2}} \right] \\ &= 1.9 \times 10^{-16} \frac{M_\odot}{\text{yr}} \left[\frac{\rho_s}{M_\odot/\text{pc}^3} \right] \left[\frac{\text{g cm}^{-3}}{\rho} \right]^{1/3} \left[\frac{M}{M_\odot} \right]^{4/3} \times \\ &\quad \times \left[\frac{\text{km s}^{-1}}{\langle V^2 \rangle^{1/2}} \right] \end{aligned} \quad (8)$$

Here ρ_s is the stellar mass density in the vicinity of the black hole. It is assumed that at very large distances from the black hole the stars have a Maxwellian distribution of velocities with an r.m.s. velocity $\langle V^2 \rangle^{1/2}$. In this derivation I have assumed that the mass and radius of a typical star broken apart by the black hole are much smaller than the mass and tidal radius of the black hole. I have also made use of the fact that the escape velocity at a distance R_T from the black hole is very much greater than $\langle V^2 \rangle^{1/2}$.

If all the gas produced in the breakup of the stars is accreted by the black hole integrating equation (8) suggests that the time required for a black hole to grow from a mass M to a mass M_c is

$$\begin{aligned} t_D &= 1.7 \times 10^{15} \text{ yr} \left[\frac{M_\odot \text{ pc}^{-3}}{\rho_s} \right] \left[\frac{M_\odot}{M} \right]^{1/3} \left[1 - \left(\frac{M}{M_c} \right)^{1/3} \right] \times \\ &\quad \times \left[\frac{\langle V^2 \rangle^{1/2}}{\text{km s}^{-1}} \right] \end{aligned} \quad (9)$$

Here I have assumed solar type stars so that $\rho = 1.41 \text{ g cm}^{-3}$. Table 1 shows t_D as a function of M for a galactic nucleus in which $\rho_s = 10^6 M_\odot \text{ pc}^{-3}$ and $\langle V^2 \rangle^{1/2} = 225 \text{ km s}^{-1}$, the observed velocity dispersion in the nucleus of M31 (ref. 7). The table also gives the luminosity, L , in units of the Eddington luminosity, L_E . Here I have used $L = 0.2 (dM/dt) c^2$. I note that at a fixed M , $(t_D)^{-1} \propto L \propto \rho_s / \langle V^2 \rangle^{1/2}$.

Thus simple scaling gives L and t_D for a galactic nucleus with any other value of ρ_s and $\langle V^2 \rangle^{1/2}$. It is not likely that $\langle V^2 \rangle^{1/2}$ differs very much from one nucleus to another, but there are sizable differences in ρ_s . Assuming $M/L = 10 M_\odot / L_\odot$, the data of Kinman⁸ indicate that $\rho_s \simeq 10^5 M_\odot \text{ pc}^{-3}$ within 1 pc of the nucleus of M31. For the nucleus of our Galaxy infrared observations⁹ indicate that $\rho_s \simeq 7 \times 10^6 M_\odot \text{ pc}^{-3}$ within 0.5 pc of its centre. Schwarzschild¹⁰ finds indirect evidence of $\rho_s = 2.2 \times 10^8 M_\odot \text{ pc}^{-3}$ within 3.5 pc of the nucleus of

the Seyfert galaxy NGC4151. For a black hole to have had enough time to grow to $M_c = 3 \times 10^8 M_\odot$ within the age of the Universe (which I assume to be 1.2×10^{10} yr), the initial mass of the black hole had to exceed $M_i \simeq 10^7 M_\odot$ in the nucleus of M31, $M_i \sim 10^2 M_\odot$ in the nucleus of our Galaxy, and $M_i \lesssim 1 M_\odot$ in the nucleus of NGC4151. The very low value of M_i for NGC4151 suggests that Schwarzschild's estimate for ρ_s may be somewhat large. From the values of L/L_E in Table 1, a black hole in the nucleus of M31 could never break up stars fast enough to allow it to radiate at the Eddington limit. In our Galaxy a black hole would radiate at the Eddington limit when its mass is equal or greater than $M_E = 10^7 M_\odot$; for NGC4151 this occurs when $M_E = 10^2 M_\odot$. When $M > M_E$ the breakup of stars by the black hole produces gas faster than the Eddington limit allows it to be accreted by the black hole. In these cases, we may expect the surplus gas to be driven out of the nucleus by radiation pressure. Such outpouring of gas is observed in several Seyferts. This mass loss can be prodigious. If $\rho_s = 10^7 M_\odot \text{ pc}^{-3}$, the mass loss rate approaches $12.5 M_\odot \text{ yr}^{-1}$ as M approaches M_c . The mass loss rate as well as the luminosity would then drop effectively to zero for $M > M_c$.

If present ideas on the evolution of massive upper main sequence stars are correct, we may reasonably expect black holes with masses of about $M_i = 10 M_\odot$ to form in almost every galactic nucleus. Such black holes would have had sufficient time in our Universe to grow to $M_c = 3 \times 10^8 M_\odot$ if the stellar density in the galactic nucleus exceeds $(\rho_s)_0 = 1.5 \times 10^7 M_\odot \text{ pc}^{-3}$ (Table 1). This watershed density is very sharply defined. If ρ_s is even 2–3 times less dense than this, the minimum mass of a seed black hole which has sufficient time to grow to M_c in the age of the Universe increases sharply to 10^2 – $10^3 M_\odot$ which is much greater than the masses of black holes formed by normal stellar evolution. If $\rho_s > 2$ – $3 (\rho_s)_0$, almost any black hole has time to grow to M_c . Thus any QSO or Seyfert nuclei present today are likely to have a stellar density of about $1.5 \times 10^7 M_\odot \text{ pc}^{-3}$ or slightly greater. At this density the black hole radiates at the Eddington limit for $M > M_E = 10^5 M_\odot$. If $\rho_s \geq 10^8$ – $10^9 M_\odot \text{ pc}^{-3}$ any black hole with $M > 10 M_\odot$ would grow at the rate governed by the Eddington limit. These black holes would have reached $M = M_c$ at about $Z = 2.8$ in the observable Universe. Thus there should be a maximum in QSO activity at this epoch followed by a rapid decline for lower values of Z . This is consistent with observations⁷.

Two refinements may increase somewhat the rate at which stars are swallowed by the black hole. One complication is the possible formation of a high density stellar cusp around a black hole embedded in a dense stellar nucleus¹¹. Another is the shrinking of the entire stellar nucleus as its stars are captured by the black hole. How this arises can be seen by considering the effect of these captures on a typical star in the nucleus. Some of the stars captured by the black hole are in orbits in which, on the average, they are further from the black hole than the test star. At some point in time each of these stars crosses inside the orbit of the test star never to return. Thus, as these stars are accreted by the black hole, the test star and the other remaining stars in the nucleus feel a progressively increasing gravitational potential. To satisfy the virial theorem, the velocity dispersion of the stars in the nucleus must increase. This added kinetic energy is supplied by a net shrinking of the effective radius, R_N , of the galactic nucleus. The increase in the velocity dispersion of the stars in the nucleus would by itself decrease the rate of accretion by the black hole, but this is more than compensated by the increased stellar density in the nucleus. I note that $\langle V^2 \rangle \propto R_N^{-1}$ while $\rho_s \propto R_N^{-3}$ (actually, $\rho_s \simeq R_N^{-2}$ when stars accreted by the black hole are considered). From equation (8), this results in a net increase in the rate of accretion by the black hole compared to the case where we ignore both the increased density and the increased velocity dispersion in the galactic nucleus. This effect can only become important as the mass of the black hole approaches that of the galactic nucleus or $M \sim 10^8 M_\odot$.

Orbit diffusion and relaxation time

In the previous section, particularly in the derivation of equation (8), I made the implicit assumption that the rate of orbit diffusion resulting from the random gravitational encounters between stars is sufficiently fast that at any given time the number of stars with orbits that pass within the Roche radius, R_T of the black hole is nearly the same as it would be if there was no breakup of these stars. This is true as long as the total mass M_n of the stars in the galactic nucleus is large compared to the mass M of the black hole. In the absence of orbit diffusion, the velocity distribution of the stars at a given point in the nucleus would be isotropic (if the stellar distribution is relaxed) except for those velocity directions corresponding to orbits which cross R_T . On average, the fraction, F_v , of the velocity directions occupied by these orbits is the fraction of all stars in the nucleus that have orbits which intersect R_T . Thus as long as $F_v \lesssim 1$, a very small amount of velocity randomisation is sufficient to fill in these small holes in the otherwise isotropic velocity distribution and consequently to repopulate the orbits that cross R_T . In other words the time necessary to repopulate these orbits is very small compared to the relaxation time¹²,

$$t_R = V^3 / 4\pi G^2 m^2 n \ln N \quad (10)$$

which is the time necessary to completely rerandomise the velocities. In the typical galactic nucleus of interest, $V = 225 \text{ km s}^{-1}$, $n = 10^7 \text{ stars pc}^{-3}$, $m = 1 M_\odot$, $M_n = 10^8 M_\odot$, and $N = M_n/m = 10^8$. In this case $t_R = 3 \times 10^8 \text{ yr}$.

The rate of orbit diffusion is sufficiently large to result in there being no significant depletion of the stars with orbits that cross R_T as long as the characteristic time for the consumption of all the stars in the galactic nucleus by the black hole is greater than the relaxation time; that is, the diffusion rate is sufficient as long as $M_n/|dM_n/dt| > t_R$. If $M_n \sim 10^8 M_\odot$, this condition is satisfied until the mass of the black hole has grown to $M \sim M_c$. Thus the assumption of an adequate rate of orbit diffusion implicit in the previous section is valid.

A massive black hole at the galactic centre?

Any such object would have to be very modest to produce no more than the observed nonstellar radiation emitted from the nucleus. If gas accretion by this object produces all the ionising radiation required to maintain the H II region at the galactic centre, the luminosity of this radiation is $L_i = 1.9 \times 10^8 L_\odot$ (ref. 13) if the energy per photon is 2 rydberg. From Table 1, with $\rho_s = 7 \times 10^6 M_\odot \text{ pc}^{-3}$, this requires a black hole mass $M \simeq 4 \times 10^4 M_\odot$. Equation (3) requires that the radius of the cloud of gas and dust produced by the breakup of stars by a black hole of this mass be $\langle r \rangle \simeq 10^{-3} \text{ pc}$. This is at least consistent with the observed "point" infrared source at the galactic centre which has a radius of less than 0.1 pc and a luminosity of $3 \times 10^5 L_\odot$. (ref. 8) $M \simeq 4 \times 10^4 M_\odot$ is likely to be a firm upper limit on M . The ionisation could be due to something else. If M is this large, it would only take another $1.5 \times 10^9 \text{ yr}$ to grow to M_c . It is unlikely (but of course not impossible) that we would catch the black hole just at this crucial stage in its growth. A more troublesome point is the fact that the initial mass of this object had to be about $10^2 M_\odot$ to have reached $M = 4 \times 10^4$ by the present epoch. This initial mass seems rather large from the standpoint of stellar evolution. If the luminosity of the infrared source results from the accretion of stars broken up by the central black hole, its mass must be at least $M \simeq 3 \times 10^2 M_\odot$. This is probably the minimum mass of the central black hole. The initial mass of this black hole need only have been slightly greater than $10 M_\odot$ to have allowed it to grow to $3 \times 10^2 M_\odot$ in $1.2 \times 10^{10} \text{ yr}$. It will take another 10^{10} yr for such a black hole to grow to $M = M_c$.

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Dredged basalts from the mid-oceanic ridge north of Iceland

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An examination of the concentrations of the incompatible elements in tholeiites from the Kolbeinsey Ridge indicates that plume magmas have probably not overflowed into that area from Iceland or Jan Mayen Island. The results also show that the chemistry of plume magmas may be intimately related to regional geochemical patterns, and it is possible that Iceland represents a 'weak spot', or decompression focus, which causes complex flow patterns within and from the asthenosphere.

WE present here petrological data on dredge samples recovered from the mid-oceanic Kolbeinsey Ridge north of Iceland. Most of the materials described were recovered during two cruises in 1972-73 in the North Atlantic.

Iceland and Jan Mayen Island represent subaerial expressions of recent volcanism associated with the Iceland Plateau and Kolbeinsey Ridge. Iceland consists of an extensive, largely tholeiitic, flood-basalt plateau built up in about the last 30 Myr and cut by three neovolcanic zones containing tholeiitic, alkalic and other basalt types¹⁻³. Its great volumes of lava, their high discharge rates and relatively high contents of K, Ti, P, rare earth elements (REE), ⁸⁷Sr/⁸⁶Sr and so on have been considered expressions of a hot, rising mantle plume^{2,4-6}.

The older Faeroes and East Greenland basalts also have K, P, and Ti values comparable to those found in Iceland⁷, and this may be⁸ the result of plume activity in the early Tertiary.

Jan Mayen Island consists of ankaramites, alkali basalts and their differentiates together with their explosive equivalents; tholeiitic flows are unknown. Subaerial material only dates back to 0.5 Myr BP but construction of the island's base could be assigned to the mid-Tertiary or early Pleistocene^{9,10}. A plume origin has also been proposed for Jan Mayen Island¹¹. The two islands lie at the limits of the Iceland Plateau, a generally smooth (presumably basaltic) platform at a depth of less than 1 km (refs 12 and 13). Transecting this plateau is the Kolbeinsey Ridge, an accretion centre erupting basalts at its axis and with minimal sedimentary cover¹³⁻¹⁵. Possible extensions of recent Icelandic and Jan Mayen plume activity may thus be sought along the Kolbeinsey Ridge.

The Kolbeinsey Ridge (66° 30'N-71° 50'N) extends, with a generally NNE trend, for a distance of approximately 550 km from Iceland to the Jan Mayen Fracture Zone and cuts the Icelandic Plateau at depths of less than 1,300 m (Fig. 1). The ridge begins as a subdued N-S trending rift valley and corresponding magnetic anomaly which gradually develops on the outer northern Icelandic Shelf. The shallow ridge axis surfaces at the islet of Kolbeinsey, 67° 08'N (ref. 16). North of Kolbeinsey Islet as far as the Spar Fracture Zone (69°N) the ridge strikes N 28°E and has a step-like structure lacking a central rift valley,

it is similar in morphology to the northern Reykjanes Ridge. In addition, this ridge segment also resembles the Reykjanes Ridge in spreading rate, free-air and Bouguer anomalies, and magnetic anomalies¹⁴. Remanent magnetisation values on drilled cores from pillow basalts from this ridge segment obtained by our shipboard spinner magnetometer averaged 0.05 e.m.u., similar to the average values found for pillow basalts on the Reykjanes Ridge¹⁷. Furthermore, thin sections show phenocryst assemblages of plagioclase-olivine-clinopyroxene (dredges 7-72, 9-72, see Table 1) which are also characteristic for pillow basalts from the Reykjanes Ridge^{4,18}. Thus, the conclusion¹⁴ that this segment of the Kolbeinsey Ridge seems to be the northern counterpart of the Reykjanes Ridge is probably correct.

North of the Spar Fracture Zone, the Kolbeinsey Ridge is offset about 30 km to the east and then takes on the classical mid-oceanic ridge structure with a well developed rift valley trending N 14°E. An unnamed fracture zone at 70° 52'N delimits the southern side of Eggvin Bank, a broad seamount transected by the ridge axis. From the top of Eggvin Bank, only 50 m below the waves, the ridge gradually deepens towards the Jan Mayen Fracture Zone which reaches depths greater than 2 km.

Chemistry of Kolbeinsey Ridge tholeiites

Table 2 presents the concentrations of K₂O, TiO₂, P₂O₅ and Sr in tholeiites from the Kolbeinsey Ridge; these have been plotted against the morphology of the ridge axis (Fig. 1). Tholeiites from the ridge axis between 67° and 70° 35'N have similar low contents of the incompatible elements; low levels of K₂O, TiO₂, P₂O₅ and Sr characterise fresh mid-oceanic ridge tholeiites¹⁹⁻²¹. In detail, the levels of K₂O, P₂O₅, Sr, and to a lesser extent TiO₂, are generally lower than oceanic ridge tholeiites south of the Charlie Gibbs Fracture Zone²⁰, and are more comparable to contents of the central and southern Reykjanes Ridge^{4,18,22}.

Eggvin Bank also consists of tholeiitic basalts but their elemental concentrations deviate sharply from the 'background level' determined for the ridge to the south. Figure 1 shows that the concentrations of K₂O, TiO₂, P₂O₅ and Sr rise to a maximum at the upper parts of Eggvin Bank. This peak in no way corresponds to the presence of highly evolved or fractionated basalts: the FeO*/MgO ratio of Eggvin Bank tholeiites is comparable with the ratio for tholeiites from the adjacent ocean floor to the south. Moreover, fractional crystallisation would lead to a similar passive concentration of elements such as K and P which enter early mineral phases in negligible amounts. In fact, K₂O concentrations are increased by a factor of seven over ridge values whereas P₂O₅ is only increased twofold. The concentration of alkali and volatile elements within a high-level magma chamber²³ of Eggvin Bank is a possibility. Alternatively, the lower TiO₂/P₂O₅ ratio of these summit tholeiites

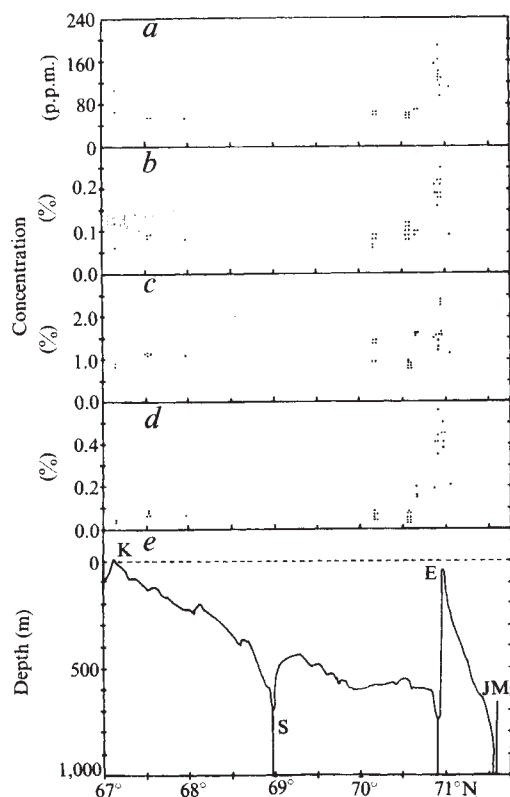


Fig. 1 Geochemical (a-d) and topographic (e) profiles along the Kolbeinsey Ridge. Concentrations of fresh tholeiites: a, Sr; b, P_2O_5 ; c, TiO_2 ; d, K_2O . e: K, Kolbeinsey Islet¹⁶⁻²¹; E, Eggvin Bank; S, Spar Fracture Zone; JM, Jan Mayen Fracture Zone.

compared with the adjacent ocean floor tholeiites may indicate a deeper origin for the Eggvin Bank basalts²⁴.

Interesting comparisons exist between Eggvin Bank and Iceland: they are both elevated topographic features, partly bounded by fracture zones and cut by an accretion axis, and significant scatter is found in the concentrations of their incompatible elements.

Tholeiites on the southern and northern flanks of Eggvin Bank show concentrations of incompatible elements which are

intermediate between those of the ridge axis and the summit of the seamount. The decrease in the TiO_2/P_2O_5 ratio is also apparent in these intermediate samples. We have only one sample (1351-1) from the northern flank of Eggvin Bank, recovered from a depth of 400–650 m at the ridge axis. It shows no sign of approaching a more alkalic province although true alkali basalts have been recovered from the walls of the Jan Mayen Fracture Zone slightly to the north (unpublished data). It seems that this sample marks a return to ocean floor tholeiite chemistry. Schilling²⁵ has reported, however, a normalised La/Sm ratio greater than 1.0 for a ridge sample from a deeper dredge only 15' of latitude south of the Jan Mayen Fracture Zone. Certainly, our samples do not provide evidence for any southward plume overflow from the Jan Mayen province extending to our most northerly sample, about 30' of latitude south of the Jan Mayen Fracture Zone. Three samples in dredge 1350-4 are also available from just to the south of the fracture zone at 70° 52' N. They were recovered from the flank of the ridge axis and exhibit elevated concentrations of K_2O (0.15 to 0.20%). These slightly higher K_2O concentrations could reflect limited degrees of low temperature, seawater alteration and/or more potassic flank volcanism.

In conclusion, the chemistry of the most southerly samples recovered from the Kolbeinsey Ridge show no indication of approaching the postulated Icelandic mantle plume; the most northerly samples show no chemical similarities to samples from the highly alkalic Jan Mayen province. Kolbeinsey Ridge crest tholeiites, with the exception of those from Eggvin Bank, show very low concentrations of incompatible elements, reminiscent of the central and southern Reykjanes Ridge. Eggvin Bank is considered to be the summit of a tholeiitic seamount sequence with enhanced contents of K_2O , TiO_2 , P_2O_5 and Sr.

Compositional gradient

We have assembled concentrations of K_2O for tholeiites from the Charlie Gibbs Fracture Zone at about 52° 46' N (Fig. 2) through the presumed Iceland plume and along the Kolbeinsey Ridge to the Jan Mayen Fracture Zone at about 71° 50' N, a distance of about 2,200 km. There is a certain degree of symmetry north and south of Iceland both in the elevation of the ridge axis and also in its structural, geophysical and petrographic character. Large sections of this geochemical profile remain unsampled, though some of the critical sections are reasonably well described. With the exception of the samples recovered from Eggvin Bank and the Minia Seamount just east of the ridge

Table 1 Dredge stations, depth of recovery and rock description

Dredge no.	Latitude and longitude	Depth (m)	Locality	Rock description
D7-72	67° 32.3' N 18° 31.2' W	445-467	Ridge crest	Pillow basalts. 10-20% vesicles (≤ 1.5 cm across). 5-10% plagioclase phenocrysts (≤ 3 mm across) + smaller clinopyroxene and olivine crystals. Devitrified vesicular basalt; 15% micropenocrysts of plagioclase + olivine + clinopyroxene.
D9-72	67° 58.5' N 18° 23.6' W	576-622	Ridge crest	Large pillow basalt fragments. Rare olivine phenocrysts + micropenocrysts of olivine + plagioclase.
D9-73	70° 10.0' N 15° 15.6' W	1,098-1,281	Rift valley	Pillow fragments with hyaloclastic upper surfaces. 1-10% phenocrysts of plagioclase (≤ 5 mm across) + rarer olivine.
D30-73	70° 34.4' N 14° 41.4' W	1,061-1,171	Rift valley	Pillow fragments; < 1 % vesicles. Few olivine and plagioclase phenocrysts
D1350-4†	70° 39.6' N 14° 18.7' W	1,000-1,400	Ridge axis flank	Single basalt fragment; $<$ detached pillow tops. Basalt fragment has 5% cavities (≤ 1 cm across) + 10% zoned and resorbed plagioclase phenocrysts + rare clinopyroxene and olivine phenocrysts.
D9-73	70° 52.6' N 13° 44.4' W	1,098-1,281	North wall of the fracture zone at junction with ridge axis	Angular vesicular basalt fragments. 5-30% vesicles (≤ 2 cm across). 10-20% phenocrysts of plagioclase + olivine $\pm$ clinopyroxene, all ≤ 3 mm across.
D28-73	70° 55.9' N 12° 59.4' W	128	Eggvin Bank	Vesicular basalts + tuff fragments. Basalts have 10-50% vesicles (≤ 1 cm across); ~ 10 % amygdaloids and up to 5% plagioclase clusters $\pm$ olivine $\pm$ clinopyroxene.
D29-73	70° 55.9' N 12° 59.4' W	55-110	Eggvin Bank	Angular pillow basalt fragment. Irregular vesicles (≤ 1 cm across). Few per cent of plagioclase micropenocrysts up to 1 mm across.
D1351-1†	71° 03.3' N 12° 58.4' W	400-650	Ridge axis	

*Thin section studies, as well as chemical analyses, indicate that the samples reported here are fresh and unaltered.

†Samples provided by Dr A. Sharaskin, Vernadskiy Institute, Moscow, from cruise of RV Akademik Kurchatov, summer 1973.

Table 2 Incompatible element concentrations in tholeiites from the Kolbeinsey Ridge

Dredge no.	No. of samples	K ₂ O concentration (%)		TiO ₂ concentration (%)		P ₂ O ₅ concentration (%)		Sr concentration (p.p.m.)	
		Average	Range	Average	Range	Average	Range	Average	Range
Kolbeinsey Islet*	3	0.036	0.03–0.04	0.67	0.66–0.68	0.09	0.06–0.12	80	65–110
D7–72	4	0.076	0.069–0.084	0.88	0.86–0.90	0.09	0.08–0.09	55	55–55
D9–72	1	0.065		0.86		0.08		55	
D7–73	6	0.063	0.041–0.088	1.02	0.76–1.16	0.08	0.06–0.09	65	60–65
D30–73	12	0.047	0.023–0.080	0.72	0.68–0.75	0.10	0.08–0.12	60	55–65
D1350–4	3	0.17	0.15–0.20	1.26	1.24–1.29	0.10	0.09–0.10	70	70–70
D9–73	1	0.19		1.20		0.21		120	
D28–73	5	0.44	0.35–0.56	1.14	0.97–1.27	0.19	0.16–0.22	145	125–185
D29–73	5	0.44	0.38–0.50	1.63	1.25–1.91	0.21	0.18–0.25	120	95–130
D1351–1	1	0.21		0.91		0.09		115	

* Refs 16 and 21.

axis, and D1350–4 from the ridge flank, all materials are from the ridge axis.

All samples chosen for study were fresh. Thin sections indicated that alteration was limited to slight discoloration of plagioclase and olivine phenocrysts; grains of chlorite, iddingite and clay minerals were not observed. Whole rock water contents did not exceed 0.7%. Zonal variation in the concentrations of K₂O was observed in several basalt pillows. For example, samples from D30–73 show their highest K₂O concentrations in the intermediate variolitic zone (0.078–0.079%), compared with the glassy margins (0.036–0.044%) and cores (0.027%).

There is some correlation between the topographic profile and the K₂O concentrations in the tholeiites. Three main divisions of the K₂O profile may be distinguished: First, the great expanse of ocean floor, about 55% of the profile, where K₂O values range from about 0.03 to 0.11% K₂O; second, the Icelandic geochemical anomaly, where the uncertain line of best fit rises to a maximum of about 0.29%, and where the scatter of potash concentrations is at a maximum. The seamounts of Minia and Eggvin both at the extreme limits of this profile and close to major fracture zones also represent scattered high-concentration potash zones, although much narrower than Iceland. Lastly, there is a mixed belt for the northern Reykjanes Ridge, where K₂O concentrations rise from oceanic to Icelandic levels. There is some indication of a 'plateau' in the trend, corresponding to the southern insular shelf of Iceland. Certainly, the K₂O contents of tholeiites reported from this shelf show no clear increase towards Iceland, although extending over 20° of latitude²⁶. From the available evidence it can be seen that an extensive mixed zone is not present (Fig. 2) on the northern shelf of Iceland. This shelf is cut by the Tjörnes Fracture Zone which offsets the spreading axis by about 100 km (ref. 27) and may act as a barrier to any northward flow of the asthenosphere²⁵.

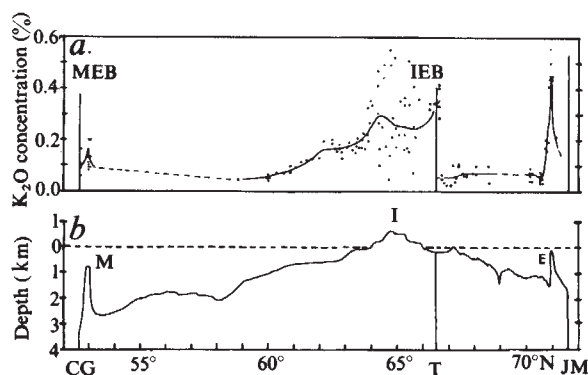


Fig. 2 K₂O concentration (a) and topographic (b) profiles along the mid-oceanic ridge between Charlie Gibbs (CG) and Jan Mayen (JM) Fracture Zones. a, K₂O contents from fresh tholeiites^{16,18,21,22,26,35}; Major element boundary (MEB) from Campsie *et al.*¹⁸ and incompatible element boundary (IEB) from both Schilling *et al.*²⁶ and this study. b, M, Minia Seamount; E, Eggvin Bank; I, Iceland; T, Tjörnes Fracture Zone.

Of particular interest is the fact that the low K concentrations of the ocean floor tholeiites to the north and south of Iceland are shared by Iceland 'plume' tholeiites. The average K₂O concentrations of Icelandic tholeiites, both from the Tertiary plateau and the neovolcanic zones, are about 0.25% (unpublished compilation), and although this value is higher than that of the adjacent ridge tholeiites, it lies within the worldwide range of K₂O concentrations for fresh, ocean ridge tholeiites^{20,21}.

In contrast to Iceland and its environs, K₂O concentrations for the Galapagos plume tholeiites average about 0.36%, and the surrounding ocean floor tholeiites average close to 0.14% (refs 28 and 29). Thus, K₂O concentrations for tholeiites of the Icelandic region, regardless of their genesis, are about 70% of those for tholeiites of the Galapagos plume and its environs. It follows that the geochemistry of plume magmas is to some extent integrated into the geochemical pattern of a broad region, and does not simply represent an independent magma type derived from independent deeper regions of the mantle, as implied by Morgan⁶. Perhaps a fairly constant factor, additive or multiplied, will convert the K₂O concentrations of the plume to normal regional concentrations.

Relationship between the plume and surrounding crust

There is one possibility whereby the Iceland 'plume' may be interrelated with the surrounding ocean floor. The Iceland area may have begun at a 'weak spot' rather than at a 'hot spot'. Such 'weak spots' may be located close to triple junctions or perhaps at the intersection of spreading axes and pre-existing tectonic lineaments, such as that delineated by the trend of the Faeroe–Iceland–Greenland ridge.

Localised decompression would lead to more extensive melting and increased basaltic discharge. Basalts generated at this focus of decompression would be equilibrated at shallower levels than normal, explaining the generally higher FeO*/MgO ratios of the basalts and also their lower eruptive temperatures³⁰. Further supplies of basaltic material would be sucked into this lower pressure region through the low velocity zone, preferentially bringing the more mobile incompatible elements with them¹⁸. This transfer of material may lead to subsidence of the oceanic crust. There may be little or no basalt replenishment because some fracture zones in this region may mark deep barriers to asthenospheric flow. Therefore, any basalts generated in the regions of greatest subsidence will have low concentrations of incompatible elements and higher concentrations of refractory MgO, giving lower FeO*/MgO values. The amounts of K, P, and so on transferred to the decompression zone may depend on the regional geochemical levels, and on the rate and degree of flow. Excess volumes of basalts may be discharged away from Iceland at shallow crustal levels and may be mixed with normal oceanic tholeiites within elongated magma chambers⁴. The available evidence suggests that while the Iceland landmass is rising³, the seafloor to the north has subsided^{31–34}.

The cruises on which we collected our samples were a co-operative effort between the Ocean Study Group, the Mineralogical and Zoological museums, Copenhagen, the Museum of

Natural History, Reykjavik, and the US Naval Oceanographic Office. We thank Professor A. Noe-Nygaard, and N. Hald of the Mineralogical Museum, Copenhagen; G. L. Johnson of USNO, and the officers and crew of the USNS Lynch for their support. Further, we thank Dr A. Sharaskin of the Vernadskiy Institute, Moscow and the staff of the Soviet R. V. Akademik Kurchatov for providing some of the samples. Finally we express our appreciation to Erik Larsen and the Laboratory for Electrophysics, Technical University of Denmark, Lyngby for the design and construction of the shipboard magnetometer.

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Some evidence of chronology and palaeoecology of Sterkfontein, Swartkrans and Kromdraai from the fossil Bovidae

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Bovid fossil remains suggest a tentative chronology for the seven cave assemblages, from Sterkfontein, Swartkrans and Kromdraai, from which they are derived. They also suggest that, during the almost 2 Myr of South African hominid and faunal Pleistocene history encompassed by this succession of assemblages, certain changes in vegetation cover and cave accumulation patterns may have occurred in the Sterkfontein Valley.

THE cranial bovid material derives from the following subdivisions, or site units, of Sterkfontein, Swartkrans and Kromdraai, all situated in the Sterkfontein Valley (see Fig. 37 in ref. 1) near Krugersdorp, South Africa: STS, Sterkfontein type locality; SE, West Pit of the Sterkfontein extension locality; D16, Sterkfontein rubble dump 16; SKa, Swartkrans assemblage from primary² (formerly pink¹) breccia; SKb, Swartkrans assemblage from (1) secondary² (formerly brown²) breccia, and from (2) fills of channels forming at a relatively late stage through both primary and secondary breccias; KA, Kromdraai faunal site; KB, Kromdraai australopithecine site. A brief summary of some currently available ideas on the hominid and cultural associations of these site units is shown in Fig. 2 and the bovid species identified at these site units are shown in Table 1.

Chronology

Much of the bovid material was found to correspond taxonomically to a greater or lesser extent with material from other sites (discussed in ref. 3). Figure 1 shows the temporal sequence of the site units, and its possible correspondence with other African sites and with the absolute time scale, as suggested by these bovid data. The most notable changes from previously accepted

chronological evaluations are as follows. It is clear that Swartkrans fossils include a substantial component that may be considerably later than the assemblage associated with australopithecines, and that Swartkrans cannot, as in the past, be considered as one unit in studies of this kind. The present bovid data also suggest that KA, KB and SE should not be included in the Swartkrans span, as has been variously done in the past, but that they may belong to the succeeding Cornelia span. Note that the present study was uninformative as to how far back in time STS and Makapansgat Limeworks may have extended³. These positions in Fig. 1 represent upper limits only.

Vegetation cover and climate

It is assumed here, as previously⁸, that the Alcelaphini and Antilopini are as a whole, among bovid tribes, indicative of open plains and grassland environments. The validity of such an assumption in this particular study, especially its application to unusual fossil forms like *Makapania*, has been discussed³. The fact that the percentages in Fig. 2 never fall below 51% seems to indicate that the past environments, corresponding to these assemblages, as a group fall distinctly into the vegetationally more 'open' part of the spectrum of known habitats. Seen from a broad perspective, therefore, these bovid data may corroborate the view¹¹ that each of the breccias in question basically represents colluvial sediments compatible only with an incomplete mat of vegetation. Figure 2, however, suggests differences within this overall environmental characterisation. The most notable difference lies between STS and the group formed by SKa, KA, SE and, less certainly, KB (the difference in percentages of STS and SKa is statistically significant³). It cannot be suggested that the smooth curve in Fig. 2 mirrors an equally smooth, simple environmental trend during the Pleistocene in the Sterkfontein Valley. Some quite substantial fluctuations may not be included in this small sampling of the whole period. Figure 2, however, may broadly represent an overall trend in the area, and probably further afield. It is likely that during most of the Middle Pleisto-

Table 1 Minimum numbers of individuals in bovid species at each of site units STS, SKa, KA, SE, KB, SKb and D16. Taxonomy as in ref. 3

	STS	SKa	KA	SE	KB	SKb	D16
Tribe: Alcelaphini							
Gp Ia: <i>Damaliscus</i> cf. <i>dorcas</i>				3		9	11
Gp Ib: <i>D. sp.</i> 2 (<i>niro</i> ?)	1			4		14	5
Gp Ic: <i>D. sp.</i> 1 or <i>Parmularius</i> sp.	7	2	32	1			
Gp II: Medium-sized alcelaphines	7	24	12	5		6	1
Gp III: cf. <i>Connocchaetes taurinus</i> lineage	1	16	5	1	3	2	1
Gp IV: cf. <i>Megalotragus</i> sp.	1	5	2				
Tribe: Hippotragini							
<i>Hippotragus</i> cf. <i>niger</i>						9	4
<i>H. cf. equinus</i>	2		1				
<i>Hippotragini</i> (?)	8	1	1	1			
Tribe: Reduncini							
cf. <i>Kobus ellipsiprymnus</i>						2	
<i>Redunca</i> cf. <i>arundinum</i>	1	1	1				
Tribe: Peleini							
<i>Pelea</i> cf. <i>capreolus</i>		2	3			7	3
Tribe: Antilopini							
<i>Antidorcas</i> cf. <i>australis</i> and <i>A. cf. marsupialis</i>						10	2
<i>A. cf. recki</i>	3	13	13	3	2		
<i>A. bondi</i>	1	7	1	2	2	63	7
cf. <i>Gazella vanhoepeni</i>	1	11					
<i>Gazella</i> sp.					1		
Gen. et sp. indet. (Antilopini or Neotragini)		3					
Tribe: Neotragini							
<i>Oreotragus</i> cf. <i>oreotragus</i>						2	
<i>O. cf. major</i>		1		1		1	
<i>Raphicerus</i> cf. <i>campestris</i>						5	2
cf. <i>Raphicerus</i> sp.			1			1	
<i>Ourebia</i> cf. <i>ourebi</i>						3	3
Tribe: Bovini							
<i>Syncerus</i> sp.	1	4	2				
Tribe: Tragelaphini							
<i>Tragelaphus</i> cf. <i>scriptus</i>		2	1			2	2
<i>T. cf. strepsiceros</i>		5	5			3	
<i>T. sp. aff. angasi</i>	1	1					
<i>Taurotragus</i> cf. <i>oryx</i>			2	1		1	1
Tribe: Ovibovini							
cf. <i>Makapania</i> sp.		4					
<i>Makapania</i> cf. <i>broomi</i>	8			1			
Uncertain sedis					1		
Totals	43	102	87	23	9	140	42

Minimum numbers of individuals, rather than the basic fossil numbers available in ref. 3, are used. This is in accordance with general practice in work on other Transvaal cave fauna (see also ref. 4).

cene an open grassland environment predominated. The onset of this open phase (in the broad sense and allowing for oscillations) succeeded a period of greater bush cover, although open country had also been prevalent during this period, as represented by STS and probably the Makapansgat Limeworks. It has been mentioned previously⁸ that either rainfall and/or temperature changes are likely to be responsible for vegetation cover changes such as that inferred between STS and succeeding Sterkfontein Valley phases; and that an explanation of the present bovid data invoking rainfall even minimally would seem to contradict Brain's findings that a general increase in rainfall, with minor fluctuations, occurred in the Sterkfontein Valley from the time of STS onwards.

There is agreement from several sources^{12,13} that a general faunal change took place in South Africa between STS and SKa times. The bovid data suggest that an environmental change may have been responsible for the faunal change, at least in the Sterkfontein Valley. It is thus not entirely idle speculation to consider whether the change from gracile australopithecines at STS and Makapansgat Limeworks to robust forms at SKa and KB may be environmentally correlated. Published opinions on fossil hominid taxonomy range from those that place all Transvaal australopithecines into one species, possibly in chronosubspecies¹⁴, to those that see them in separate species¹⁵ or separate genera¹⁶. If the latter view were taken one could note

that the basic premises of Robinson's¹⁶ dietary hypothesis would fit an open grassland-robust correlation very well, although the last step (based on ref. 1) of his argument would not; that is, seeing the 'crushing, grinding' robust vegetarian in a (slightly) wetter and more luxuriant environment than the gracile omnivore. If one views the SKa and KB australopithecines as differently adapted to their gracile counterparts, perhaps one should consider whether their musculature was so massive and, the molars proportionally so large, because their 'vegetables' were of the tough grassland type. The long-adapted Phase 1 hominid, or 'small object' vegetarian of Jolly's 'seed-eaters' hypothesis¹⁷ fits well in this respect. Of course, if these robust and gracile forms were considered to be similarly adapted¹⁴, with the often cited skull and dentition differences merely a consequence of overall size difference, the only admissible correlation would be between vegetationally more open (and drier and/or colder) environments and larger body size.

Is it a coincidence that an East African proliferation of bovids adapted to open country, apparently occurring during or above member G at Omo (A. W. Gentry, unpublished) and in the *M. andrewsi* zone at East Rudolf (J. M. Harris, unpublished), seems to coincide temporally with the analogous South African development? It could be possible that such a change in faunal tribal representation, mirroring a change in environmental exploitation, marked the boundary between a Sterkfontein span (or similar term) and a succeeding span not only in the Sterkfontein Valley, but elsewhere in Africa as well.

Possible accumulation patterns

Some tentative suggestions as to the possible predominant causes of accumulation of these bovid assemblages are shown in Fig. 3. To evaluate such aspects in this particular case, where only adult-juvenile and weight data of bovids from a series of cave accumulations are available, the following model was set up: Stage 1: bovids are killed by carnivore or hominid predators (hominid hunters are included as a subset of the set of predators) in the vicinity of the cave, or die a natural death in or near the cave.

Stage 2: remains of dead animals are brought into the cave eventually to constitute one or the other of the following:

(a) Primary assemblage: brought into the cave, which serves as a lair or shelter, by the primary predator(s); unlikely to contain a low percentage of juveniles; and if one predatory pattern predominates, most individuals should fall into a restricted body weight range.

(b) Secondary assemblage: brought into the cave by scavengers (for example, hyaena, hominid) and/or collectors (for example, porcupine, hominid); likely to contain a low percentage of juveniles; and the body weight distribution is likely to have a high variance.

A fuller discussion of this suggested scheme, and of the validity of the implied characteristics of primary and secondary assemblages, is contained in ref. 3. Most of the data in Fig. 3 do not lend themselves to a statistical approach, and what follows is a number of suggestions as to possible dominant accumulation patterns.

Juveniles of the smaller species are often eaten totally, or almost totally, by predators¹⁸ and consequently have less chance of being preserved in fossil accumulations. One can therefore expect fossil juvenile-adult ratios to be heavily biased towards adults. With this consideration in mind juvenile percentages in STS, KA and D16 particularly, and in SKa, KB and SKb (Fig. 3a), seem high in comparison with those recorded in live populations^{19,20,21}. In fact it looks as if juveniles might have been selected in preference to adults in these cases; and it is likely that they represent primary, or predominantly primary assemblages. SE clearly differs from the other site units in this respect.

STS. The most frequently occurring species are of medium to large size—weight class I is entirely absent. The exceptionally high average weight (Fig. 3c) indicates predators that were

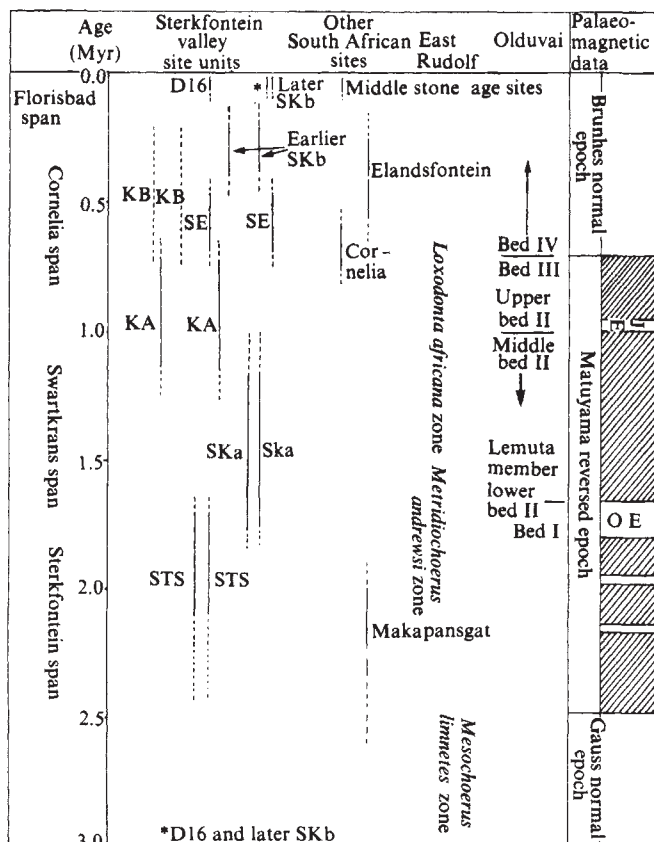


Fig. 1 The bovid data suggest chronological placement of the Sterkfontein Valley site units somewhere along the vertical lines drawn. Palaeomagnetic and Olduvai data approximated from ref. 5, East Rudolf faunal zones from refs 6 and 7. JE, Jaramillo Event; OE, Olduvai Event.

specialised on large prey. It cannot be ruled out that *Australopithecus* contributed in the role of scavenger, but his main 'contribution' is likely to have been as carnivore prey. STS carnivore remains include *Megantereon*, a true sabre-tooth cat, *Dinofelis*, a false sabre-tooth cat, and a leopard^{22,23}. As individuals of the size preferred as prey by modern leopards¹⁸ are in the minority, it seems likely that the major predators were the sabre-tooths. Ewer²⁴ has pointed out that, as a result of highly efficient carnassial shear, these carnivores were well adapted to the slicing of meat. The premolars, however, were so reduced that the animals could only have crushed the smaller bones, leaving the niche for bone-crushing specialists, like hyaenids, wide open. Brain²⁵ further elaborated on this idea: "Regrettably little is known about the behaviour of South

African sabre-tooths and it is uncertain whether they were dominant to the associated hyaenas or whether they were forced to retreat with their prey into the seclusion of caves or trees".¹⁷ During much of the accumulation period the STS cave may have been such a sabre-tooth lair. Whole kills, or parts of larger kills may have been brought to the cave for peaceful consumption by the predator and its brood.

SKa and KA. Brain²⁶ has given convincing reasons why the predominant contribution to the SKa assemblage should have come from leopards. In comparing remains from KA and KB (C. K. Brain, personal communication) he has noted the following. Although the more complete specimens in the former assemblage suggest that KA may have been a carnivore lair, the extremely fragmented KB bone remains may represent food remains of hominid hunters. The bovid assemblages from SKa and KA seem to support these ideas. In both cases the majority of individuals include high percentages of juveniles and fall into a restricted medium weight range, suggesting dominant predators of a somewhat smaller prey adaptation than those at STS (in the case of KA possibly the large lion-like felid present in that assemblage). As at STS the percentages of juveniles do not increase in the larger weight categories (Fig. 3b). In both cases adult bovids in the largest weight class, as well as sabre-tooth remains, are present, suggesting that these predators may have played a secondary role.

KB, SKb, D16. The last three site units in Fig. 3 have several features in common. Like the three earlier carnivore assemblages they have high overall juvenile percentages, although they diverge sharply from STS, SKa and KA in Fig. 3b and c. All three later assemblages are strongly dominated by species of low weight. In each case the few large-sized individuals include a distinctly higher percentage of juveniles than do the lower weight classes. The pattern is again strongly suggestive of predation, this time by a predator specialised on small prey. The three site units have more in common: They have been placed last in the chronological sequence on taxonomic grounds. The presence of both *Homo* and stone tools is either known, or inferred for reasons independent of their similarity in Fig. 3, in all three site units. The obvious conclusion is that KB, SKb and D16 each represent (predominantly, if not entirely) the food remains of hominid hunters. Note that while scavenging probably did play a part, Fig. 3a-c are unanimous in stressing an overriding hunting pattern.

SE. The presence at this site unit of numerous stone tools suggests that the Sterkfontein cave during SE times may have been an occupation site of hominids. Yet the pattern at SE (Fig. 3) is quite different from those presented by the inferred hominid hunting remains from KB, SKb and D16. In fact, its markedly low juvenile percentage and the broad, platykurtic distribution of only 23 individuals throughout all weight classes, suggest that SE represents the only markedly non-primary

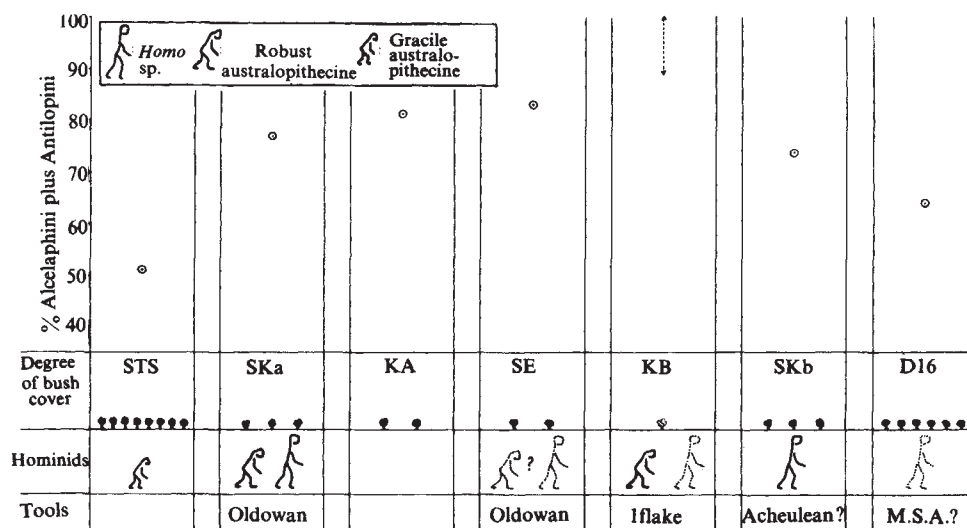


Fig. 2 The percentage constituted by the added minimum numbers of alcelaphine and antilopine individuals of the total minimum number of bovid individuals, per site unit, is suggestive of the degree of bush cover during deposition (as crudely represented by the small trees). Solid line hominid figures express widely accepted taxonomic evaluations; dotted lines signify in the cases of KB and D16 that the presence of *Homo* is only inferred, in the case of SE that uncertainty exists as to which hominid(s) is (are) present, but that *Homo* may be included⁹. Cultural associations of SKa and SE as in ref. 10, of KB as in ref. 1, of SKb according to Brain (unpublished), of D16 inferred. Percentage cover: eight trees, 51-55%; seven, 56-60% and so on; one, 86-90%; none, 91-100%.

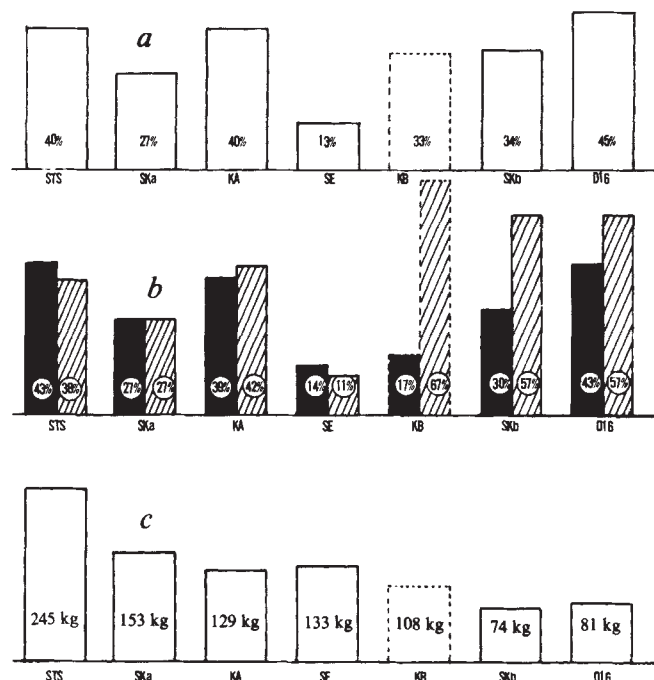


Fig. 3 Changes in average body weight and in juvenile proportions from site unit to site unit. Weight classes I–IV of Bovidae as defined in ref. 4; all data for a–c from ref. 3. *a*, Percentage juveniles of total minimum number of individuals in each site unit. *b*, Percentage juveniles present in combined weight classes I and II (solid columns, <80 kg per individual) on the one hand, and III and IV (hatched columns, >80 kg per individual) on the other, in each site unit. *c*, Estimated average weight (kg) per individual, in each site unit.

assemblage among those considered in this study. An hypothesis that the SE toolmaker was a scavenger would fit the available data very well. If the extensively fragmented SE bovid remains were indeed the discards of meals scavenged from carnivore kills, one would not expect a high percentage of juveniles, nor an increased sampling of juveniles in higher weight categories (Fig. 3*b*), although the average weight of what is consumed may still be as high as that of a primary assemblage of carnivore remains (Fig. 3*c*).

The pattern presented by the bovid remains is consistent with their chronological succession. In the earlier three assemblages

there is strong evidence of carnivore occupation of the Krugersdorp caves, and such parameters as those used here agree consistently from site unit to site unit. Thereafter the only evidence available here indicates (at least predominant) hominid occupation of caves in the same places that were previously dominated by carnivores. Were carnivores dominant to earlier hominids, but ousted by their more advanced descendants, in the competitive search for shelter in the Sterkfontein Valley? Certainly the site units here investigated can be divided into an earlier carnivore occupation phase and a later hominid occupation phase. Within the latter, the hominid quest for animal protein may have first led to scavenging, and only later to hunting. If the present interpretation of bovid remains is correct, it would support the conclusion of a number of authors that tools were being fashioned before attainment of the hunting adaptation. SE tools, serving to procure vegetable foods and to prepare scavenged meat, may have been the hominid preadaptation for the hunting which is evident in the later site units.

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Structural invariants in protein folding

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An analysis of 15 protein structures indicates: First, the loss of accessible surface area by monomeric proteins on folding—proportional to hydrophobic energy—is a simple function of molecular weight; second, the proportion of polar groups forming intramolecular hydrogen bonds is constant; and third, protein interiors are closely packed, each residue occupying the same volume as it does in crystals of amino acids.

THE aesthetic shock of the first protein structures is expressed in Kendrew's contemporary description of myoglobin¹ as: "... almost nothing but a complicated set of rods [of poly-

peptide] sometimes going straight for a distance then turning a corner and going off in a new direction. ... much more complicated and irregular than most of the early theories of the structure of proteins had suggested".

Although structural similarities between proteins of different sequence but similar functions have become apparent, the image of protein structure as a disordered and complex assembly of pieces of secondary structure has continued to the present day.

At the same time as the first protein structures became known a simple qualitative theory for the energies responsible for maintaining protein structure was described, principally

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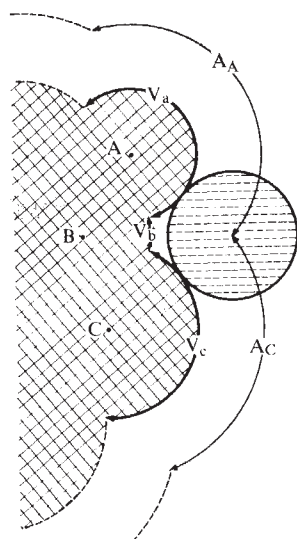


Fig. 1 Two-dimensional illustrations of the concept of accessible surface area—the area over which the centre of a water molecule can be placed while retaining van der Waals' contact with that protein atom and not penetrating any other atom. Three atoms, A, B, and C have the van der Waals' envelopes V_A , V_B and V_C which define the surface of the protein. Atoms A and C have the accessible surface represented by the arcs A_A and A_C ; A and C sterically prevent any contact between water molecule shown and atom B which therefore has no accessible surface.

by Kauzmann². Essentially, this stated that when a protein folds the gain in hydrophobic energy, as a result of the reduction in the number of non-polar contacts with water, compensates for the loss of chain configurational entropy; and that polar groups on the interior of the protein form hydrogen bonds³.

Here I show that, in place of chain topology, structural descriptions are used which are directly related to energy terms—accessible surface areas, hydrogen bonds and residue volumes—there are simple equations or expressions which quite accurately describe protein structures.

The atomic coordinates of 15 proteins were used: lysozyme, α -chymotrypsin, insulin, rubredoxin, porcine trypsin inhibitor (PTI), high potential iron protein (HIP), calcium binding protein (CBP), ribonuclease S, staphylococcal nuclease, papain, concanavalin A (con A), subtilisin, thermolysin, carboxypeptidase A and lactate dehydrogenase (LDH). The

atomic coordinates of the first two proteins were energy refined by Levitt^{4,5} and I carried out a preliminary energy refinement of the coordinates of the last 10.

The structures of haem proteins are not described by some of the relationships derived below and will be dealt with elsewhere.

Accessible surface area of proteins

The term "accessible surface area" has been introduced by Lee and Richards⁶ to describe the area over which contact between protein atoms and water molecules can occur. For a particular atom, this term is defined as the area over which the centre of a water molecule can be placed while retaining van der Waals' contact with that atom and not penetrating any other protein atom (Fig. 1). Atomic accessible surface areas were calculated by Lee and Richards⁶ for lysozyme, ribonuclease S and myoglobin, and by Shrake and Rupley⁷ for lysozyme and insulin. It was later shown that in proteins the hydrophobic free energies are directly related to the accessible surface area of both polar and non-polar groups⁸. Here I show that the hydrophobic energy of proteins—proportional to the loss of accessible surface area that occurs on folding—is related to its molecular weight by a simple equation.

In the calculations described below the following abbreviations are used

A_S , The accessible surface area of the folded native protein.
 A_T , The accessible surface area the protein would have if it unfolded and assumed an extended conformation with the side chains *trans*.

A_B , The accessible surface area that is buried when the protein folds; equal to $A_T - A_S$.

F_{AS} , The proportion of the proteins' accessible surface that is buried when it folds: equal to A_B/A_T .

Accessible surface areas were found using the method described by Lee and Richards⁶ and a computer program written by Levitt (personal communication). Hydrogen atoms are not considered individually but included in the van der Waals' radii used for non-hydrogen atoms. The van der Waals' radii used, oxygen 1.40 Å, trigonal nitrogen 1.65 Å, tetrahedral nitrogen 1.50 Å, tetrahedral carbon 1.87 Å, trigonal carbon 1.76 Å, sulphur 1.85 Å and water 1.40 Å, were based on the intermolecular contact distances observed between non-hydrogen atoms in the accurate crystal structure analysis of amino acids (Koetzle, personal communication). Accessible surface areas of individual residues, R, were calculated using the peptide Gly-R-Gly which has an extended β conformation ($\Phi = -140^\circ$, $\Psi = 135^\circ$) and the side chains fully extended

Table 1 Accessible surface area and proportion of polar groups forming intramolecular hydrogen bonds in 15 proteins

Protein	Molecular weight	A_T	A_S	A_B	Calculated A_B	F_{AS}	F_{HB}
Insulin*	5,787	8,380	3,440	4,940	(5,230)	0.59	0.37
Rubredoxin†	6,108	8,670	3,320	5,350	5,530	0.62	0.36
PTI	6,158	9,440	3,700	5,740	5,580	0.61	0.36
HIP	8,976	13,380	4,600	8,780	8,330	0.66	0.46
CBP	11,489	16,740	5,740	11,000	10,890	0.66	0.52
Ribonuclease S	13,690	19,840	6,550	13,290	13,210	0.67	0.42
Lysozyme	14,814	20,560	6,480	14,080	13,880	0.68	0.48
Staphylococcal nuclease‡	15,973	23,800	7,840	15,960	15,690	0.67	0.48
Papain	23,423	33,850	9,210	24,640	24,360	0.73	0.42
α -ChymotrypsinΦ	24,608	35,900	10,130	25,770	25,820	0.72	0.44
Con A*	25,578	37,030	10,230	26,800	(27,030)	0.72	0.40
Subtilisin	27,534	40,090	9,790	30,300	29,510	0.76	0.51
Thermolysin	34,334	49,410	11,570	37,840	38,600	0.77	0.49
Carboxypeptidase	34,409	49,870	11,260	38,610	38,710	0.77	0.50
LDH*	36,470	53,080	17,060	36,020	(41,610)	0.68	0.42

Areas are expressed in Å². Calculated A_B = the value of A_B given by equation (3). F_{AS} = the proportion of the protein's accessible surface area that is buried when it folds: equal to A_B/A_T . F_{HB} = the proportion of protein polar groups forming main chain-main chain or side chain-main chain hydrogen bonds.

*Calculation carried out on an isolated monomer.

†Molecular weight of the sequence found by the X-ray analysis (ref. 23).

‡Calculations do not include the terminal residues 143–149 which are not seen in the Fourier rays.

ΦCalculations do not include residues 10–16 which are not seen in the Fourier map.

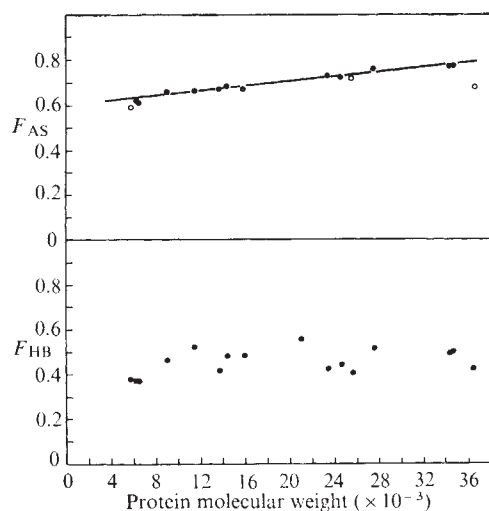


Fig. 2 upper, F_{AS} , the proportion of the potential accessible surface area a protein buries when it folds, and lower, F_{HB} , the proportion of protein polar groups forming one or more main chain-main chain or main chain side chain hydrogen bonds; plotted against protein molecular weight. The F_{AS} values for oligomeric proteins are represented by open circles.

($\chi_1 = -120^\circ$, $\chi_{n+1} = 180^\circ$). The values of residue accessible surface areas were similar to those found by Shrake and Rupley⁷, though they used slightly different van der Waals' radii and averaged over a number of different residue conformations.

In Table 1, the values for A_T , A_S and A_B are listed for 15 proteins. A_T values were found by summing the residue accessible surface areas for each residue present in the protein.

As might be expected, A_T is proportional to molecular weight (M). The expression

$$A_T = 1.44M \quad (1)$$

gives values in $\text{\AA}^2$ within 1% for 12 of the proteins considered and within 3% for the rest. In Fig. 2a, the value of $F_{AS} = (A_B/A_T)$ is plotted against molecular weight for each protein. The points on this graph can be divided into two sorts: the oligomeric proteins, insulin, concanavalin A and lactate dehydrogenase,

and the rest, which are monomeric proteins with between 54 and 316 residues. A least squares treatment shows that for the monomeric proteins F_{AS} is linearly related to molecular weight by the equation

$$F_{AS} = 0.595 + 0.536M \times 10^{-5} \quad (2)$$

The protein accessible surface area which is buried on folding, A_B , is $A_T \times F_{AS}$. Therefore,

$$A_B = 0.859M + 0.774M^2 \times 10^{-5} \quad (3)$$

This equation gives values for A_B in $\text{\AA}^2$ to within at least 3% of those observed (Table 1) for all the monomeric proteins. Nozaki and Tanford⁹ calculated, from the free energy of transfer of amino acids from water to ethanol or dioxane, the hydrophobicity values of 10 side chains; and if, in the manner described previously⁸, these values are equated with the side chain accessible surface areas calculated here, it is found that for each $\text{\AA}^2$ removed from contact with water there is a free energy gain of 20 calorie (the value given in ref. 8 using the side chain accessible surface areas of Lee and Richards, was 24 calorie). If the transfer is from water to hydrocarbon solvents, instead of to ethanol, free energy values are about 20% higher¹⁰. Therefore taking the hydrophobicity of amino acid residues to be 24 calorie per $\text{\AA}^2$ the hydrophobic contribution to the free energy of folding, E_{HP} , in calories is (from equation (3))

$$E_{HP} = 21M + 19M^2 \times 10^{-5} \quad (4)$$

The application of equation (2) to proteins larger than those considered here is obviously limited. The little information so far available (for example, refs 11–14) indicates that the structures of large globular proteins consist of quite distinct domains of a size similar to the molecules considered here and that these domains can by themselves retain a functional structure. This suggests that for large globular proteins F_{AS} is essentially constant.

There are three oligomeric proteins in the sample considered here: insulin, con A, and lactate dehydrogenase. The observed values of F_{AS} for insulin and con A, 0.59 and 0.72 respectively, are smaller, though not significantly, than the values calculated

Table 2 Volume occupied by residues in the interior of 9 proteins

Residue	Total no. in the proteins	No. buried*	Average volume (V_R) of buried residue ($\text{\AA}^3$)	S. D. of V_R ($\text{\AA}^3$)	†Residue crystal volume equal to amino acid volume less $11.1\text{\AA}^3$
Val	163	91	141.7	8.4	143.4
Ala	183	71	91.5	6.7	96.6
Ile	106	69	168.8	9.8	169.7
Gly	160	60	66.4	4.7	66.5
Leu	138	57	167.9	10.2	—
Ser	190	46	99.1	7.4	102.2
Thr	128	32	122.1	6.7	124.3
Phe	60	29	203.4	10.3	—
Asp	117	17	124.5	7.7	122.0
Cys	34	16	105.6	6.0	108.7
Pro	67	16	129.3	7.3	124.4
Met	28	14	170.8	8.9	176.1
Tyr	98	13	203.6	9.6	201.7
Glu	65	13	155.1	11.4	143.9
Asn	116	12	135.2	10.1	—
Trp	39	9	237.6	13.6	—
His	43	8	167.3	7.4	166.3
Lys	119	5	171.3	6.8	—
Gln	80	5	161.1	13.0	148.0
Cyh	10	4	117.7	4.9	123.1
Arg	63	0	—	—	—

The nine proteins are: CBP, ribonuclease S, lysozyme, papain, α -chymotrypsin, subtilisin, carboxypeptidase, thermolysin and LDH.

*A residue is defined as buried if 5% or less of its potential accessible surface area is available to solvent contact.

†11.1 $\text{\AA}^3$ is the volume lost by an amino acid on becoming a residue. The value used here was found by comparing the crystal volumes of glycine and glycyglycine, and is identical to the value in Cohn and Edsall¹²—11.0 $\text{\AA}^3$ —determined by solution studies. No accurate unit cell dimensions were found for Leu, Phe, Asn, Trp and Lys.

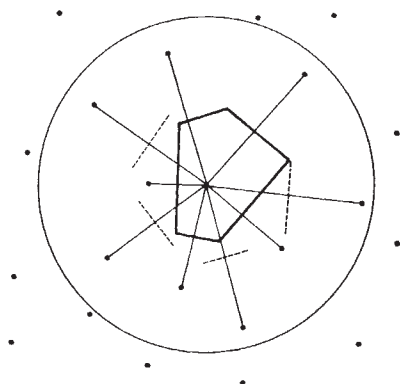


Fig. 3 Two-dimensional illustration of Richards' method of finding the volume occupied by an atom in a protein. Vectors are made from the atom to all neighbours within a radius of 6.5 Å. Planes perpendicular to each of these vectors are then constructed at a point related to the van der Waals' radii of the two atoms forming the vector. The smallest polyhedron so constructed—the Voronoi polyhedron—is the volume occupied by the atom (see refs 19 and 20).

from equation (3), 0.63 and 0.73. The observed F_{AS} value for LDH, 0.68, however, is very significantly smaller than the calculated value, 0.79. Subunit contacts involve large hydrophobic free energies⁹, and so these observed F_{AS} values suggest, the subunit contacts formed in oligomeric proteins may allow the subunits to have a more open structure than that found for monomeric proteins of the same size.

Intramolecular hydrogen bonds in proteins

The free energy of intramolecular protein hydrogen bonds as compared with those between water and protein is unknown. Experimental values for the difference in enthalpy are small^{15,16}. We could expect the entropy contribution to favour protein-protein hydrogen bonding as this would release bound water molecules (as occurs on the melting of ice where each molecule gains 18 e.u.).

Protein hydrogen bonds were found by using a computer program (Levitt, personal communication) which, given a list of non-hydrogen coordinates, adds hydrogens in positions fixed by the known stereochemistry of H-X-W bond angles and lengths, and then prints out Z-Y...H-X arrangements where Y is a hydrogen bond acceptor, H-X a donor and the geometry is satisfactory for hydrogen bond formation: a hydrogen bond was accepted if the distance Y...X was 3.5 Å or less, the angle Z-Y...X more than 90°, and the angle Y...H-X more than 150°.

Hydrogen bonds were listed in this way for the 15 proteins. In Fig. 2b the proportion of the polar groups that were found to form one or more main chain-main chain or main chain-side chain hydrogen bonds, F_{HB} , is plotted against the protein's molecular weight. The precise absolute values for F_{HB} are not significant as they depend on the geometrical criteria used to determine hydrogen bonds. The differences between F_{HB} values for proteins of molecular weight greater than 8,900 are not significant since the number of polar groups which correctly form hydrogen bonds depends on the quality of Fourier map and the subjection of the initial coordinates to Diamond model building, and real space, difference Fourier and energy refinement⁵. This is particularly true of the few groups forming side chain-side chain hydrogen bonds. For this reason and because many of them have a transient existence^{17,18}, they were not included.

From Fig. 2b, it is unambiguously clear that, for those proteins considered here that have a molecular weight over 8,900, the proportion of polar groups forming intramolecular hydrogen bonds is essentially constant and close to 0.5.

For most globular proteins the number of polar groups present is proportional to molecular weight: of the 15 considered here

$M/38.8$ gives the number of polar groups to within 3% except for lysozyme (4%) and ribonuclease (5%). This implies that to a good approximation the contribution of hydrogen bonds to the free energy of protein folding is simply proportional to molecular weight.

Volume occupied by residues in the interior of proteins

Globular proteins are distinguished from fibrous proteins and other two-dimensional biological structures by the ability of the pieces of secondary structure from which they are formed to associate and give a stable three-dimensional structure. I shall discuss briefly the nature of the molecular interactions that occur between pieces of secondary structure.

The problem can be put in terms of the question: how well do pieces of secondary structure recognise each other? The quantitative answer can be found by calculating the volumes occupied by residues in the protein interior. Large residue volumes (low density) would imply that the recognition was poor and the association similar to that which occurs in micelles, while a small volume (high density) could mean that the recognition is precise and similar to that which occurs in crystalline solids. Residue volumes are directly related to packing energies and conformational entropies.

The calculation of residue volumes is part of the larger problem of protein density and the most rigorous approach to this is undoubtedly that of Richards¹⁹ who adopted the approach used by Bernal and Finney²⁰ to describe the structure of liquids, and introduced the use of Voronoi polyhedra to calculate the volumes occupied by protein atoms. The volume occupied by a protein atom is found, by first constructing vectors to all neighbouring atoms, second, at a point along each vector related to the van der Waals' radii constructing a perpendicular plane, and third, finding the smallest polyhedron described by these planes (Fig. 3). The method and its implementation is fully described in refs 19 and 20. By its application Richards showed the interior of lysozyme and ribonuclease to have a packing density of 0.75, similar to that of small organic crystals.

I have calculated the volumes occupied by residues buried in the interior of the calcium binding protein, ribonuclease S, lysozyme, subtilisin, α -chymotrypsin, carboxypeptidase A, papain, thermolysin and lactate dehydrogenase. As a working definition, proteins were taken to be buried if less than 5% of their potential accessible surface was available to solvent contact. Atomic volumes were found by using the B version of Richards' computer program¹⁹ and residue volumes found by summing the value calculated for each atom. The results are summarised in Table 2 which also lists the volumes which residues occupy in crystals of amino acids.

Richards observed that protein interiors are close packed¹⁹. From Table 2 this observation can be extended to state that, in general, the volume occupied by a particular residue in the interior of a protein is constant (the standard deviations are ~6%); and that it is the same as that occupied by the residue part of the amino acid in its crystal form. Thus the "oil drop" model of protein structure with its implication of the micelle-like association of pieces of secondary structure is incorrect, while the description of proteins as "molecular crystals"²¹ is accurate. The close packing of protein interiors prevents water molecules being trapped in non-polar cavities, maximises the packing energy and implies that the stable association of pieces of protein secondary structure depends on the existence of interfaces that can close pack and are large enough in area to give sufficient hydrophobic energy. This can probably be generalised to apply to recognition processes that occur between other kinds of biological molecules.

Of the buried residues listed in Table 2 those with non-polar side chains (Val, Ala, Ile, Leu, Gly, Phe and Pro) make up 67% by number (and 66% by volume) of the total while those with polar side chains make up 33%. Experimental solution studies indicate that as long as unchanged polar groups retain their

hydrogen bonds their hydrophobicity is similar to that of non-polar groups⁸; and, indeed, Lee and Richards found that when ribonuclease S, lysozyme and myoglobin go from an extended conformation to the native structure the reduction that occurs in the accessible surface area of polar and non-polar groups is the same⁹. But the necessity for, and geometrical requirements of, hydrogen bond formation by interior polar side chains severely limits the possibilities of formation of close packed molecules by pieces of secondary structure. Twenty-six per cent of the serine and threonine residues are buried but only 10% of the asparagines and 6% of the glutamines, both of which have two side chain polar groups that need to form hydrogen bonds (Table 2). The high proportion of non-polar side chains that occur in protein interiors essentially results from the necessity of forming a close packed structure in which nearly all buried polar groups form hydrogen bonds.

It follows that structural homology between proteins of different amino acid sequence is essentially a packing phenomenon.

Protein folding

The folding problem of a globular protein is how to gain enough energy from hydrophobicity, hydrogen bonds and van der Waal's interactions to overcome the loss of configurational entropy and the steric strain that occurs in the folded state. The calculations described here show that the structural solution to at least part of this problem is described by a set of simple expressions, and raise the question: are there regularities present in the topology of the folded chain—in the amount of secondary structure and

the manner in which it is assembled—which are responsible for these simple expressions?

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Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids

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Corticosteroids as well as non-steroid anti-inflammatory drugs inhibit the prostaglandin-mediated vasodilatation accompanying lipolysis in subcutaneous fat. Whereas the non-steroids produce their effect by inhibition of prostaglandin synthesis, however, corticosteroids inhibit their release. This mechanism may be the basis of some actions of corticosteroids in inflammation, in the gastric mucosa and in the CNS.

A PROSTAGLANDIN (PG), probably E₂ (refs 1 and 2) is a mediator of functional vasodilatation (vasodilatation accompanying activity) in subcutaneous adipose tissue. In 1971 it was discovered³ that non-steroid anti-inflammatory agents such as aspirin and indomethacin prevented PG formation by inhibition of PG synthetase. It was subsequently shown that such anti-inflammatory agents inhibit PG formation and functional vasodilatation in subcutaneous fat but do not prevent lipolysis⁴. Therefore, it seems likely that these actions are the result of inhibition of PG synthetase activity in adipose tissue. We have now shown that functional vasodilatation in fat is inhibited not only by non-steroid anti-inflammatory drugs of the aspirin type,

but by the anti-inflammatory steroids hydrocortisone, betamethasone and prednisolone as well.

Inhibition of vasodilatation

The method used was that described earlier¹ in which the venous outflow from the epigastric adipose depot of rabbits was measured directly with a drop counter and fat mobilisation was induced by a close-arterial infusion of adrenocorticotrophic hormone (ACTH₁₋₂₄). This lipolysis was accompanied by PGE₂ formation in the fat tissue and dilatation of the blood vessels giving an increased blood flow.

When hydrocortisone (2 nmol min⁻¹ to 2 μmol min⁻¹) as hemisuccinate was infused into the fat depot immediately before the infusion of ACTH₁₋₂₄, the vasodilatation was greatly reduced or abolished. The blood steroid level during the infusion was about the same as that in a rheumatoid patient on steroid therapy. Figure 1 shows the percentage inhibition of the vasodilatation produced after activating the fat tissue with a close-arterial infusion of ACTH₁₋₂₄ (1 μg min⁻¹), brought about by 15 min infusions of various concentrations of hydrocortisone.

The first interpretation of this finding was that anti-inflammatory steroids as well as aspirin-like drugs inhibit PG synthetase in adipose tissue. It has been suggested that PG biosynthesis in

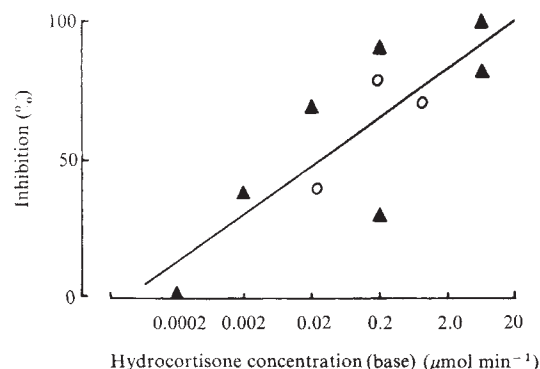


Fig. 1 The effect of hydrocortisone (as hemisuccinate) on the vasodilatation in subcutaneous adipose tissue ($\blacktriangle$), and on the output of a prostaglandin-like substance into the venous effluent blood ($\circ$), during lipolysis brought about by a 5 min close-arterial infusion of ACTH_{1-24} . Each point represents the result from one rabbit. The inhibition is plotted as a percentage of control and the concentration of hydrocortisone in $\mu\text{mol min}^{-1}$ is plotted on a log scale. The line is the regression line calculated from the points plotting the inhibition of vasodilatation.

human skin is inhibited by corticosteroids⁵. The same research group⁶, however, working with a microsomal fraction from skin, have more recently agreed with other groups who found that anti-inflammatory steroids do not inhibit the PG synthetase activity of homogenates prepared from lungs, spleen or other tissues. When we studied prostaglandin formation and leakage from another tissue, the rabbit jejunum, as described earlier⁸, our results were in agreement with theirs. We found that although indomethacin (30 nmol ml^{-1}) inhibited the leakage of PG from segments of rabbit jejunum suspended in 15 ml Krebs solution at 37°C , hydrocortisone caused no reduction. This finding implied that unlike indomethacin, hydrocortisone did not inhibit PG synthetase.

We therefore analysed further the hydrocortisone-induced inhibition of the PG-mediated functional vasodilatation in adipose tissue. We wanted to determine whether hydrocortisone was inhibiting the vasodilatation by inhibiting lipolysis itself, or by antagonising the action of PG, or by preventing its formation, or by interfering in some way with the release of PG after its formation.

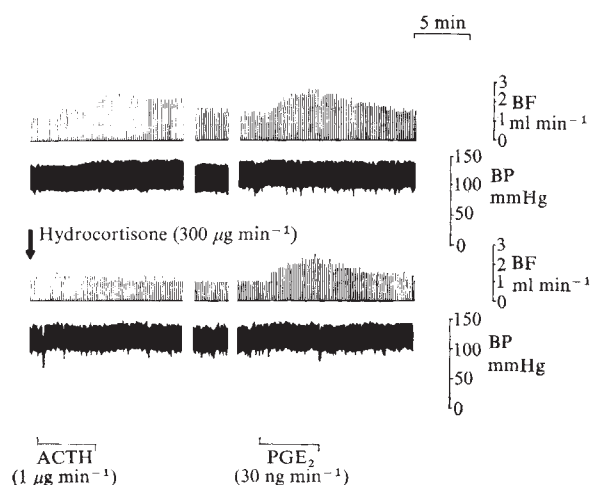


Fig. 2 Record of venous outflow (BF) upper record, from the right epigastric fat pad and arterial blood pressure (BP) in a 4.5 kg female New Zealand White rabbit. Responses to 5 min close-arterial infusions of ACTH_{1-24} ($1 \mu\text{g min}^{-1}$) and PGE_2 (30 ng min^{-1}). Upper tracings before and lower tracings after close-arterial infusion of hydrocortisone hemisuccinate ($300 \mu\text{g min}^{-1}$) (equivalent to hydrocortisone $600 \text{ nmol min}^{-1}$). The gaps between each block of tracing is equivalent to 30 min. The increased venous outflow following ACTH_{1-24} is almost abolished whereas the response to PGE_2 is unaffected.

First, we were able to show that hydrocortisone does not inhibit lipolysis. When hydrocortisone ($600 \text{ nmol min}^{-1}$) was infused close-arterially to the epigastric fat depot immediately before the lipolytic substance ACTH ($1 \mu\text{g min}^{-1}$), the free fatty acid content of the epigastric venous blood reached a level of $3\text{--}5 \text{ meq l}^{-1}$, that is the same level reached after stimulating with ACTH_{1-24} in the absence of hydrocortisone.

Second, the inhibition of vasodilatation was not the result of a direct antagonism of the action of PG by the corticosteroids. In three experiments, infusions of hydrocortisone ($600 \text{ nmol min}^{-1}$) (the ED_{75} used to inhibit functional vasodilatation) did not inhibit the vasodilatation produced by close-arterial infusions of PGE_2 at $30\text{--}100 \text{ ng min}^{-1}$. A typical experiment is illustrated in Fig. 2. In a second series of experiments betamethasone (as disodium phosphate) given at a rate of $2\text{--}20 \text{ nmol min}^{-1}$ inhibited the vasodilatation accompanying lipolysis more than 90% whereas the vasodilatation produced by infusions of PGE_2 ($30\text{--}100 \text{ ng min}^{-1}$) was not reduced at all. In some experiments it was even potentiated.

Third, using the method of Bowery and Lewis⁴ it was possible to show that hydrocortisone did not inhibit the formation of PG in the fat tissue during lipolysis. The epigastric fat depots of both sides of the animal were isolated from the skin and abdominal wall and prepared for close-arterial injection of a fat-mobilising agent. ACTH_{1-24} ($1 \mu\text{g min}^{-1}$) was infused close-arterially to the right epigastric fat pad for 5 min, and 15 min after beginning the infusion the fat pad was excised and placed immediately into ice-cold ethanol, chopped finely and homogenised at 0°C using an Ultra Turrax homogeniser. After allowing the rabbit to recover for 1.5 h after the ACTH_{1-24} infusion,

Table 1 Prostaglandin activity in fat pad extracts in equivalents of PGE_2 ng per g tissue formed after activation with ACTH_{1-24}

Experiment	ACTH_{1-24} ($1 \mu\text{g min}^{-1}$)	Hydrocortisone ($6.0 \mu\text{mol min}^{-1}$) + ACTH_{1-24}	Indomethacin (30 nmol min^{-1}) + ACTH_{1-24}
1	38.1	45.5	
2	36.0	47.0	
3	8.0	7.1	
4	47.6		<0.25

hydrocortisone ($600 \text{ nmol min}^{-1}$) was infused close-arterially to the left fat pad for 15 min followed immediately by ACTH_{1-24} ($1 \mu\text{g min}^{-1}$) for 5 min. Again 15 min after the beginning of the infusion of ACTH_{1-24} the fat pad was excised and placed immediately into ice-cold ethanol, chopped and homogenised as before. Both fat pads were examined for prostaglandin content by extraction into ice cold ethanol and ethyl acetate at pH 3. The extracts were evaporated to dryness under reduced pressure at a temperature not exceeding 45°C . The residues were taken up in 0.1 ml ethanol and applied to thin-layer chromatographic plates using the A1 solvent system⁹. PGE_2 and $\text{PGF}_{2\alpha}$ standards were chromatographed simultaneously with the extracts and were made visible by exposure to iodine vapour. The silica gel was scraped from the thin-layer plates at 1–2 cm intervals and suspended in 1 ml Krebs solution. The PG activity of the supernatant was estimated by bioassay on the isolated rat stomach strip (RSS), rat colon (RC) and chick rectum (CR). All the PG activity from the fat pad extract appeared in fractions corresponding to the PGE_2 standard.

The results given in Table 1 show that hydrocortisone does not prevent the formation of PG when the fat tissue is activated. The increased PG content of extracts of fat pads which occurs on activation⁴ was not inhibited by hydrocortisone. On the other hand, as shown in the Table, a similar experiment confirms the earlier observation⁴ that indomethacin prevents or reduces the formation of PG.

Prostaglandin release

Since the corticosteroids inhibit the vasodilatation but not the formation of PG in the tissue, it seems possible that they prevent the release of PG from the fat cells thereby preventing their

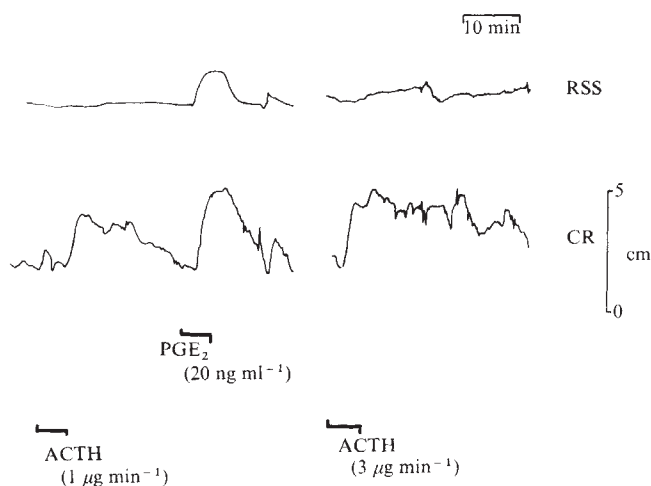


Fig. 3 The venous effluent from the epigastric fat pad of an anaesthetised New Zealand White rabbit (female, 4.3 kg) was superfused over two isolated assay tissues, rat stomach strip (RSS) and chick rectum (CR) and returned to the animal by way of a jugular vein. When PGE_2 (20 ng ml^{-1}) was infused into the blood bathing the assay tissues (IBB) both RSS and CR contracted. When ACTH_{1-24} (1 and $3 \mu\text{g min}^{-1}$) was infused close-arterially to the fat pad for 5 min a substance causing contraction of CR (approximately equivalent to PGE_2 20 ng ml^{-1}) but not RSS was released into the venous blood. In other experiments ACTH_{1-24} did not contract the assay tissues when given IBB. Vertical scale 5 cm, time 10 min.

action on the blood vessels. A suggestion that this might be true arises from a study of the release of a PG-like material from the fat pad into the blood during lipolysis. The method was to prepare the fat depot as before for recording venous outflow and for making close-arterial infusions. In this series of experiments, however, the venous blood from the fat depot was superfused over a series of isolated smooth muscle tissues, RSS, CR and RC¹⁰ before being returned to the animal. The tissues were made more specific for PG-like materials by bathing them before superfusion in a solution containing antagonists of histamine, 5-hydroxytryptamine (5-HT), acetylcholine and catecholamines and infusing these inhibitors through the lumen of CR and RC during the experiment¹¹. Figure 3 illustrates the release into the venous effluent of a substance which contracts the isolated chick rectum which is sensitive to PGs of the 'E' type. Like the vasodilatation⁴, the output of this material was reproducible when repeated stimulations by ACTH were made at hourly intervals. When the venous effluent was tested on the RSS, RC and CR and compared with responses to PGE_2 it was obvious that the substance in the venous blood was not PGE_2 , although the substance formed in the fat tissue itself is PGE_2 (ref. 2) as recently confirmed¹² using mass spectrometric analysis. Although the venous effluent blood consistently contracted CR and to a lesser extent RC, it caused little or no contraction of RSS. In addition, when the epigastric venous blood collected during lipolysis was extracted with solvent systems similar to those used for extraction of PGs¹³, a little PG-like activity was retained. The substance however, was not PGE_2 , compared with which it was more active on CR and RC than on RSS. Neither ACTH_{1-24} itself nor any substance released during infusion of ACTH_{1-24} altered the responsiveness of the isolated tissues to PG.

In further experiments of this type we have found that the output of this material during activation of the adipose tissue is prevented by previous infusion of indomethacin. This finding strongly suggests that although the material is not PGE_2 , it is a product of the PG synthetase system. This effect was observed not only with indomethacin, however, since infusions of hydrocortisone or betamethasone before the ACTH_{1-24} -induced activation of the adipose tissue also prevented the output of the PG-like material as illustrated in Fig. 4. Figure 1 shows that

when the output is plotted against concentration of hydrocortisone, the points fall along the same curve obtained from a plot of inhibition of vasodilatation.

In spite of its resemblances to PGs, the possibility exists that the chick rectum contracting substance is an unrelated new kind of chemical mediator the release of which is inhibited by both steroid and non-steroid anti-inflammatory agents.

Active transport inhibition

In summary we have found that like non-steroid anti-inflammatory drugs such as aspirin and indomethacin, anti-inflammatory steroids such as hydrocortisone, betamethasone and prednisolone inhibit the PG-mediated functional vasodilatation in subcutaneous adipose tissue. The mechanism of action of the steroids is, however, different. Like indomethacin, hydrocortisone inhibits vasodilatation, prevents the output into the blood of a PG-like substance, possibly a metabolite, and does not antagonise the vasodilator action of PG directly. But whereas indomethacin inhibits the formation of prostaglandin in the fat tissue itself, hydrocortisone does not; it therefore seems likely that hydrocortisone inhibits the release of prostaglandin. Perhaps it does this by preventing the transport of prostaglandin from inside the fat cell to the extracellular space where it would normally act on the blood vessels. This is the first indication that formation and release of PGs may be distinguishable since previously the two processes were thought to be equitable¹³.

Since the discovery that non-steroid anti-inflammatory drugs produce at least some of their anti-inflammatory effects by inhibition of PG synthetase thereby preventing the formation of PGs, it has been difficult to explain the same kind of anti-inflammatory activities of corticosteroids, since it has been demonstrated by several groups of workers that anti-inflammatory steroids do not inhibit PG synthetase.

In situations where PG formation occurs extracellularly or where the cells are damaged so much that their membranes no longer provide a barrier to the diffusion of materials, PG synthetase inhibitors would prevent the appearance of PGs. For example, in our experiments with strips of rabbit jejunum, it seems likely that PGs are probably formed during cell breakdown and their leakage from these damaged cells could not be expected to be inhibited by steroids, although their formation and therefore their leakage is inhibited by indomethacin. It has also been shown that steroids did not affect PG levels in an inflammatory exudate *in vivo*¹⁴, that is, another situation where there is cell necrosis. But Ferreira *et al.*¹⁵ found a similar result in perfused spleen where cell damage would seem unlikely,

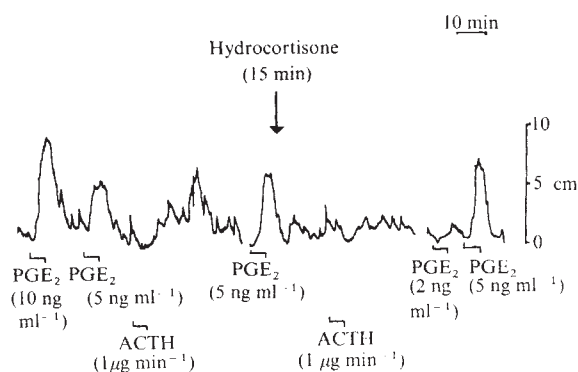


Fig. 4 The venous effluent from the fat pad from a female New Zealand White rabbit (3.4 kg) was superfused over a single CR. The tracing is arranged and labelled as in Fig. 3. In the first panel calibrating doses of PGE_2 of 5–10 ng ml^{-1} were given IBB. When ACTH_{1-24} ($1 \mu\text{g min}^{-1}$ for 5 min) was infused close-arterially to the fat pad a substance which caused a contraction of CR equivalent to 5–10 ng ml^{-1} PGE_2 was released into the venous blood. In the second panel, hydrocortisone (500 nmol) was infused close-arterially to the fat pad for 15 min immediately before ACTH_{1-24} ($1 \mu\text{g min}^{-1}$). This time there was no release of contractile substance. 1.5 h elapsed between doses of ACTH_{1-24} .

although the perfusions with Krebs solution and dextran might have caused some alterations in the cells. On the other hand, during lipolysis there is no damage to the fat cells and the release of PGs from these cells is therefore probably an active process rather than leakage. It is this 'active transport' which seems to be inhibited by corticosteroids. It seems likely that this effect on subcutaneous adipose tissue might well account for some anti-inflammatory effects of corticosteroids in skin, in particular their local vasoconstrictor activity¹⁸. The inhibition of PG release may well have further implications on the mode of action of corticosteroids on inflammatory cells and the cells of other systems like those of the reproductive tract.

Such a hypothesis needs confirmation by experiments with isolated cells. First it would be required to show that isolated fat cells synthesise PG when stimulated and that its release is inhibited by anti-inflammatory steroids. It would also be interesting to examine the effect of steroids on the release of PGs from cells which play an obvious role in inflammatory reactions, such as mast cells, leukocytes and platelets.

Until now there has been no report of a transport mechanism for the release of PG from cells; formation being synonymous with release. But some investigations have indicated that there might be an active transport mechanism for the uptake of PGs^{17,18}. After injection of ³H-labelled PG radioactivity was found to be concentrated in some tissues. In addition, PGs were found to be rapidly taken up into lungs but the parent compounds and metabolites were released slowly. On the other hand, there have been many reports of the membrane stabilising effect of anti-inflammatory steroids like hydrocortisone¹⁹. It is not clear precisely what is meant by 'stabilisation' but it might well be involved in the inhibition of PG release.

Inhibition of PG synthetase by non-steroid anti-inflammatory drugs may not only be the basis of their anti-inflammatory activity but may also be the cause of their main side effect—gastric irritation. If PGs mediate functional vasodilatation in the gastric mucosa as has been suggested²⁰, PG synthetase inhibitors would probably produce areas of ischaemia leading to necrosis. As anti-inflammatory steroids also produce gastric ulceration, it

is possible that they act in the gastric mucosa too, by inhibiting the release of PG.

Another instance where the inhibition of PG release by anti-inflammatory steroids might explain their action is in their effect in controlling fever. It has been suggested that pyrogenic fever is caused by release of PGs in the anterior hypothalamus^{21,22}. In addition, it has been shown that PG synthetase inhibitors reduce or prevent the release of PGs into the cerebral ventricles and reduce fever^{23,24}. The release of PGs in the hypothalamus may well be mediated through a similar sort of transport mechanism as that which seems to be present in adipose tissue. It would therefore be interesting to examine the effect of corticosteroids on PG release in the brain during fever since it is known that certain kinds of fever are alleviated by these agents.

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letters to nature

Silicon-rich stellar envelope?

DURING an analysis of Orion-2 data derived from a space astrophysical experiment¹, we came across a hot star with a gaseous envelope, SAO077308, of B1e type and nearly ninth magnitude. It is interesting because of an extremely strong emission line at 2,520 Å occurs in its ultraviolet spectrum. Following a series of attempts, the identification of this line with the resonance sextet of neutral silicon (mean wavelength 2,520 Å) seems probable. The components of this sextet, of almost equal strengths, are: 2,507, 2,514, 2,516, 2,519, 2,524 and 2,528 Å; the second of these lines is resonant, the others quasi-resonant in the sense that the lowest levels of these lines are located quite close to the ground level, up to 0.01–0.03 eV. This identification cannot be taken as final and needs further examination. Irrespective of possible closer definitions, however, the very presence of the emission line itself in the spectrum of this star is significant. Regardless of the true nature of the chemical element to which this line belongs, the problem of an anomalous abundance of this element cannot be overlooked.

Here we report the further somewhat conventional examination of the abnormal abundance of silicon in the envelope of the star SAO077308.

The emission line at 2,520 Å is the strongest in the spectrum of SAO077308 at least in the range from 4,000 Å to 2,300 Å; one can see its unusual strength in the densitometric recording of the spectrum of this star in Fig. 1 (the spectral resolution is nearly 15 Å at 2,500 Å). To establish the anomalous nature of the line in the spectrum of this star, a comparison with the spectrum of another well known emission star, ζ Tau, of almost the same spectral class, has been made. This is shown in Fig. 2 by collating the densitometric recordings of the same parts of both spectra near 2,500 Å.

A few spectral images with an exposure time of 7–8 min (frame F23) have been obtained for the star SAO077308. The spectral pictures of ζ Tau were obtained from Orion-2 with an exposure time of nearly 5 s (F4), that is, in conditions considerably more favourable than in the case of SAO077308, bearing in mind the inevitable deterioration of the quality of the spectrograms during long exposures as a result of the

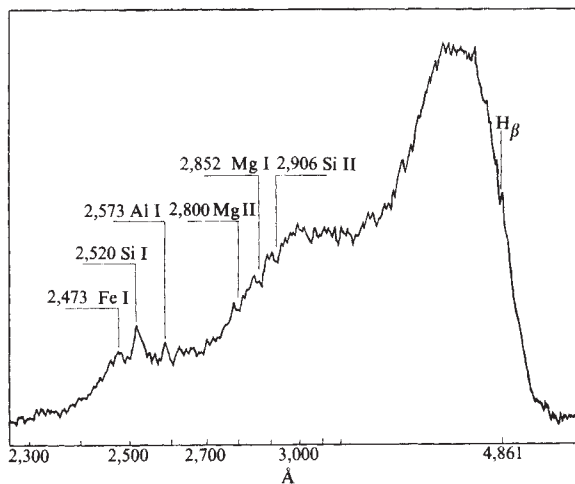


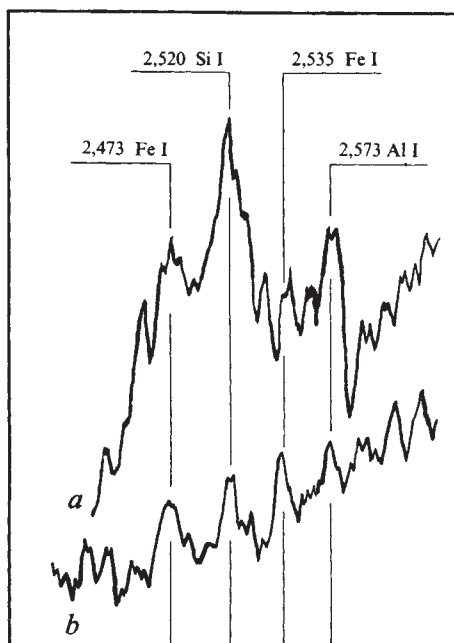
Fig. 1 Densitometric recording from the ultraviolet spectra of emission star SAO077308 in the range of wavelengths from 5,000 Å to 2,300 Å. The strongest emission line 2,520 Si I is visible.

accidental disturbances of the guide system of Orion-2. Nevertheless, the line at 2,520 Å is sharply distinguished compared with nearby emission lines in SAO077308, but this line has the same intensity as those of nearby emission lines in the spectrum of ζ Tau. The alternative assumption that this difference may be the result of the anomalously small abundance of iron (2,473 Fe I, a resonance doublet) and aluminium (2,573 Al I, a resonance triplet) in SAO077308 seems unlikely.

It is difficult to determine the relative abundance of silicon in the envelope of the star SAO077308 as compared to ζ Tau, in view of the different conditions in which the spectral images were taken. A very approximate estimate shows that the relative abundance of silicon in the envelope of SAO077308 must be at least 3–4 times, and perhaps a whole order of magnitude, larger than in the envelope of ζ Tau.

To what degree the discovered abnormal abundance of

Fig. 2 Comparison of two densitometric recordings from the spectra of the stars SAO077308 (a) and ζ Tau (b), near 2,500 Å, from which the anomalous intensity of 2,520 Si I in the case of SAO077308 is clear.



silicon is characteristic, not only of the gaseous envelope of the star SAO077308 but also of the photosphere itself, is difficult to specify without additional data. To achieve this, it is necessary to look for the absorption lines in the spectrum of the star under examination by means of ground based astrophysical observations and to compare those lines with their analogues in the spectrum of the comparison star.

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Ionospheric mid-latitude trough and the abrupt scintillation boundary

THE mid-latitude trough^{1,2} in electron density and the equatorward limit of small-scale F-region irregularity structure apparent in the scintillation of satellite and radio star signals, the scintillation boundary^{3,4}, are both features of the transition region between the high and mid-latitude ionospheres. There are broad similarities between the morphology and statistical behaviour of the trough and the irregularity boundary⁵⁻⁷ and, although the individual behaviour of the scintillation boundary and the mid-latitude trough may be related to particle precipitation patterns or plasmopause movements, there is a definite local time variation in their mean relative latitudinal positions when simultaneous observations of the two phenomena are analysed⁸. We have examined this variation in more detail, using data from simultaneous observations of trough and scintillation boundary positions.

Some 530 recordings of passes of the ionospheric beacon satellite BE-B (1964–64A) taken at Aberystwyth (52.42°N, 4.05°W) were selected from the available records for the years 1966 to 1968 inclusive. The sample analysed comprised those records which showed irregular Faraday fading rates, indicating steep gradients in total electron content, or a sharp transition from zero to full scintillation of the 40 MHz signal indicating the presence of a clear scintillation boundary. Reduction of the 40 and 41 MHz records by means of the differential Faraday technique yielded the variation of total electron content with invariant latitude, for an assumed ionospheric height of 350 km, over the approximate range 45° to 63°. These plots of total electron content were used to determine the trough latitude; for the purposes of the analysis this was taken to be that corresponding to the minimum electron content recorded in the trough. The invariant latitude, at 350 km, of the scintillation boundary was also determined. The definition of the boundary used, a mean between zero and full modulation of the signal, was of the 'abrupt' type⁹, with 95% of the sample having widths less than 3°, the width being the latitudinal range over which the scintillation depth varied from zero to full modulation of the 40 MHz signal.

The results (Fig. 1) show the contrast between the diurnal behaviour of the trough and scintillation boundary latitudes as a function of time. The trough was first observed at ~59°Λ at 1900 LMT, falling rapidly in latitude to ~55°Λ at 2300 LMT and then more slowly to reach a minimum ~54°Λ at 0600 LMT; subsequently it returned very rapidly to higher latitudes. A separate study of the gradients of the trough walls indicates that this apparent movement to higher latitudes is a filling of the equatorward margin of a stationary trough formation by solar electromagnetic radiation produced ionisation as dawn advances, rather than a true poleward motion of the trough as a whole. In contrast to trough behaviour, the scintillation boundary has been observed at all times of the day; it

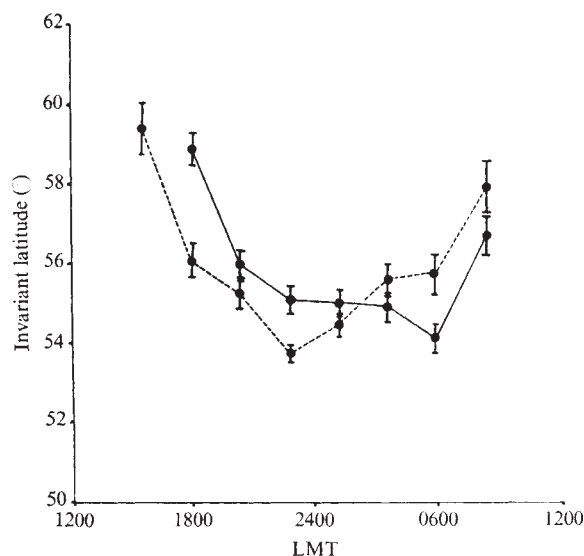


Fig. 1 Variations of the latitude of the mid-latitude trough and the scintillation boundary as functions of local mean time. The solid line represents the trough, the dashed line the scintillation boundary. The points plotted represent median values for each time interval, using only data for which the planetary magnetic index, K_p , was less than four. The bars denote the standard errors of the median values.

is found at $59.5^\circ\Lambda$ at 1600 LMT, falling steadily to a minimum at $53.5^\circ\Lambda$ by 2300 LMT and retreating more slowly to reach $\sim 58^\circ\Lambda$ by 0900 LMT. These results are in broad agreement with the mean latitudinal differences in trough and scintillation boundary positions reported previously⁸. It now seems that the differences in the relative behaviour noted earlier, based on simultaneous observations, also occur in the absolute statistical variation of these phenomena; furthermore the clear differences in the diurnal behaviour of the trough and the scintillation boundary can explain these results.

We conclude that the mid-latitude trough and the scintillation boundary, although both features of the high to mid-latitude ionosphere interface, are essentially independent having distinct diurnal behaviour patterns. It must therefore be supposed that the immediate causative mechanisms, whether in terms of particle precipitation, plasmopause behaviour, electric field effects or some more complex process involving plasma waves, are also essentially unrelated.

The movement of the scintillation boundary, reaching minimum latitude around local midnight, is similar, although displaced towards the equator, to that of the edge of the auroral oval closest to the equator. It is possible that an energy source at E-layer altitude, within the auroral oval, acts by means of electric fields or perhaps plasma waves to cause the observed irregularities which lead to scintillation. It does not seem that the trough can be related directly to reported particle precipitation patterns, but its movement shows certain similarities to that of the plasmopause, at least in the midnight to post-dawn sector. But the present results do not reveal any motion of the trough which can be related to the 'dusk bulge' in plasmopause position. We therefore conclude that one-to-one correspondence between trough and plasmopause movements may not be maintained at all times.

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Interaction of a photon with a gravitational field

It is usual to analyse the behaviour of photons in gravitational fields as if they were point particles which travel along geodesics. But by taking into account the finite spatial extent of the photon I show that it could interact with the transverse gradient of the gravitational field to give a deflection additional to the well known gravitational deflection of General Relativity. I postulate that this deflection, even though it is extremely small, could be the basis for a much more important effect in which the photon can decay into three photons (or possibly with one or more gravitons). If such a mechanism is possible it would appear as a decrease in photon energy and could be of astrophysical importance in explaining many of the anomalous redshifts that have been observed^{1,2}.

In some situations the secondary photons which are expected to have very low energies may be observed as low frequency radio waves.

For a classical electromagnetic wave its linear momentum $\mathbf{p}$ and its angular momentum $\mathbf{L}$ are given by the two volume integrals

$$\mathbf{p} = \int \epsilon \mu \mathbf{N} d\tau \quad (1)$$

$$\mathbf{L} = \int \epsilon \mu \mathbf{r} \times \mathbf{N} d\tau \quad (2)$$

where $\mathbf{N}$ is the localised Poynting vector ($\mathbf{N} = \mathbf{E} \times \mathbf{H}$).

Moller³ has shown that in General Relativity for the description of electromagnetic radiation a weak stationary gravitational field may be considered as a refractive medium with $\epsilon \mu = 1/c^2(1 - 2\chi/c^2)$ where χ is the gravitational potential. Clearly this description would be valid for many other theories of gravitation which predict the deflection of light by a massive body. Now in a region of space which is large compared to the possible extent of a photon but small compared to variations in the gravitational field, the gravitational potential can be expressed as $\chi = \chi_0 + \nabla \chi \cdot \mathbf{r}$, where $\mathbf{r}$ is vector centred in the region.

$$\mathbf{p} = \left[1 - (2\chi_0/c^2) \right] \mathbf{p}_0 - (2/c^2) \int (\nabla \chi \cdot \mathbf{r}) \mathbf{N} d\tau \quad (3)$$

where $\mathbf{p}_0$ is the momentum in a region containing no field and the first term on the right is the usual gravitational redshift, which is of no further concern here. Using the vector triple product expansion the second term can be expanded to give

$$\int (\nabla \chi \cdot \mathbf{r}) \mathbf{N} d\tau = \int (\nabla \chi \cdot \mathbf{N}) \mathbf{r} d\tau - \int \nabla \chi \times (\mathbf{r} \times \mathbf{N}) d\tau \quad (4)$$

applying these equations to a circularly polarised photon which has circular symmetry about its trajectory the first term on the

right of equation (4) is zero and equation (2) can be substituted in the second term to give

$$\mathbf{p} = [1 - (2\chi/c^2)]\mathbf{p}_0 - (2/c^2) \nabla\chi \times \mathbf{L} \quad (5)$$

Since a photon has angular momentum parallel to its trajectory equation (5) predicts an additional deflection orthogonal to both the photon trajectory and to the gradient of the gravitational field (the vector taking opposite sign for right handed and left handed polarisation). Thus for a photon that just grazes the edge of the Sun there is no change between the initial and final directions but there is a small lateral displacement. For a photon of wavelength λ this is $2GM\lambda/\pi R$ where G is the gravitational constant, M is the mass of the Sun and R is its radius. The separation between right handed and left handed polarisations of a source at the Sun is twice this value and corresponds to an angular separation of 1.5×10^{-11} arc s for $\lambda = 37$ nm. This is very much smaller than a recent⁴ upper limit of 2×10^{-3} arc s.

The angular momentum may be calculated by substitution in equation (2) but in this case the term proportional to the gradient of χ is zero because of the circular symmetry about the trajectory of the poynting vector $\mathbf{N}$. This leads to the 'paradox' that the photon spin is no longer parallel to the photon trajectory as defined in equation (5). One could imagine the reference trajectory to be defined by the photon spin and the last term in equation (5) to be the result of a perturbation caused by an interaction with the gravitational field. Havas⁵ has shown that if there is a momentum transfer, as occurs here, there is no known reason why the photon cannot split up into three or more photons. It is the conservation of spin, momentum and energy which gives the photon the characteristics of a single particle. I therefore postulate that a photon can interact with a gravitational field to produce two or more secondary photons. It seems reasonable to assume that the rate of energy loss per unit path length resulting from the production of secondary photons is proportional to the magnitude of the transverse momentum given by equation (5), and to make the proportionality constant dimensionless the path length is measured in wavelength units. Alternatively, one could argue that the distance between interactions is that which would give a lateral displacement of the order of a wavelength and each interaction produces on average an energy loss proportional to the energy of the primary photons. Both arguments lead to the same equation for the energy loss, namely

$$(1/E)\partial E/\partial l = - (K/c^2)|\nabla\chi \times \mathbf{n}| \quad (6)$$

where l is the distance measured along the photon trajectory, K is a dimensionless constant and $\mathbf{n}$ is a unit vector parallel to the photon trajectory. This decay process can be likened to the removal of a tidal strain set up in the photon by the gradient of the gravitational field and it must therefore compete with any other process that could remove the strain. In particular if there is a refractive medium present the excess momentum could be transferred to the medium without the decay process occurring. In order to estimate the constant K this postulate can be used to explain the anomalous redshift¹ observed in solar emission lines coming from the limb of the Sun. Then by integrating equation (6) the redshift may be expressed as an effective velocity v where

$$v = (KGM/cR) ((1 - \cos\theta)/\sin\theta) \quad (7)$$

where θ is the angle between the line of sight and the solar radius at the point of observation.

Since the experimental observations are subject to an unknown additional constant the 25 velocities given by Adam¹ in his Fig. 6 were fitted by the least squares method to give

$$(0.962 \pm 0.083)[(1 - \cos\theta)/\sin\theta] + (0.416 \pm 0.065) \text{ km s}^{-1}$$

Then equating the first coefficient to the constant in equation (7) gives $K = 1.51 \pm 0.13$. Although the r.m.s. residual (0.05 km s^{-1}) is small, the experimental scatter is too large to confirm the functional form of equation (7).

Since the secondary photons will have frequencies lower than the plasma frequency of the solar corona there will be virtual emission with all the energy loss being used to heat the corona. Integration of equation (7) shows that the energy loss to the solar corona measured per unit area of the photosphere is $(KGM/cR)/(\pi/2 - 1) F$, where F is total radiation flux density of the primary photons. For the Sun with $F = 6.41 \times 10^7 \text{ W m}^{-2}$ the loss to the corona is 117 W m^{-2} (with $K = 1.5$) or a total power of $7.08 \times 10^{20} \text{ W}$. At the solar minimum the estimated loss⁷ to radiation and the solar wind is 200 W m^{-2} , however, allowance must be made for conduction losses to the chromosphere, values for which vary from⁸ 50 W m^{-2} to an order of magnitude greater⁷. Athay⁸ has argued that this conduction loss may not be a genuine loss but that the energy is returned to the corona by mass motions of the chromospheric filaments. In any case these low energy photons could be an important energy source for the solar corona.

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Is the Earth tide phase lag unaffected by anelasticity?

THE dynamics of the Earth-Moon system raises the question whether the bodily tides or ocean tides in the Earth-Moon system produce most of the energy dissipation which is necessary to account for the secular retardation of the rate of rotation of the Earth. Macdonald¹ has shown that a phase lag of 2° of bodily tides is enough to account for the whole change, and in principle this phase lag could be obtained from earth tide observations. In practice, however, the determination of the phase lag of Earth tides is extremely uncertain, since the true value of the delay is influenced by the lag of recording instruments and by several disturbing effects. Pariisky² found that the mean value of the phase lag of solid Earth tides in Europe and in Asia is approximately equal to that of mentioned above, but the individual values are so much different one from another that the averaging is rather uncertain. The study reported here indicates that all of the phase lag derived from Earth tide observations may be instrumental in origin.

Miller³ investigated in detail the possibility of dissipation in shallow seas. His results hint that shallow seas are responsible for two-thirds of the dissipated energy; this numerical value, however, is so uncertain that as little as one-third or as much as

100% of the dissipated energy may be attributed to the shallow seas.

I have obtained the Earth tide phase lag in a different way, computing the phase delay in case of a supposed model of the internal structure of the Earth and of its rheological characteristics. The phase lag of crustal deformations has been computed using Molodensky's⁴ theory. As a starting point I used the homogeneous equations for the deformations of an elastic Earth of spherical symmetry⁴ leading to a set of six linear differential equations. In this equation system six unknown functions occur, these being proportional to the radial and tangential components of the displacement vector, to stress, to the tide generating potential and to its derivatives, respectively. For example, one of the functions sought for and denoted by H satisfies the following connection with the U_r radial component of the displacement vector:

$$U_r = H_n(a^{n-1}/r^n)/(W_n/g)$$

where a is the radius of the Earth, r is the distance from the centre of the Earth to the point of interest, g is acceleration due to gravity, n is the degree of harmonic development of the tidal potential W . Introducing complex expressions of the shear modulus and of the unknown functions we have

$$\bar{\mu} = \mu_0(1 + im) = \bar{\Phi}_j = \Phi_j + i\Phi_j^* = j = 1, 2, \dots, 6, \quad (1)$$

and we obtain for the imaginary parts of the corresponding functions an inhomogeneous differential equation system taking into account the effect of anelasticity too. In equation (1) μ_0 represents the real part of the shear modulus, $i = \sqrt{-1}$, m is some chosen function and Φ_j denote the functions sought for. The bulk modulus is taken here as being a real number. This means that energy dissipation in compression is much less than in shear. Solving the homogeneous differential equations relating to the real parts of the functions being sought we can compute the values of the real parts of Love numbers. The imaginary parts can be obtained using the method of variation of constants, after having obtained the solution of the problem relating to the deformations of ideally elastic Earth.

Full details of the method are published elsewhere^{4,5}; here I restrict myself to the description of results of the computations.

I assumed the Earth model of Bullen and Haddon⁶ denoted as model B_2 . I also assumed that rheological features of the Earth can be characterised by the Knopoff-Lomnitz body, that is, the Q dissipation function does not depend on the frequency of oscillations. Furthermore, it is known that in case of a Knopoff-Lomnitz body function m figuring in equation (1) is connected with Q by

$$m = Q^{-1}$$

The radial variation⁷ of the function Q is given in Table 1. Solving the equations of the deformations of the elastic Earth gives the values of the Love numbers the values included in Table 2. These values are in good agreement with those obtained from observations. On the basis of the processing of a 50,000-d long series of Earth tide measurements, Melchior⁸ gives the following values for k and h :

$$k = 0.316 \pm 0.010, \\ h = 0.637 \pm 0.016$$

After solution of the problem relating to the ideally elastic Earth, I turned to the solution of that dealing with deformations

Table 2 Computed values of the second and third order Love numbers

n	h	l	k
2	0.6335	0.0882	0.3228
3	0.2983	0.0150	0.0970

of an inelastic globe, using the Q profile given in Table 1. The imaginary parts of the Love numbers obtained are almost zero. Consequently, the phase lag of tidal deformation—which can be given, as a matter of fact, as the ratio of the imaginary and real parts of Love numbers—vanishes, and for practical purposes we have no phase lag. For example, the computed value of the phase lag of gravimetrically obtainable δ factor is only 1–2. These results suggest that at present the solid Earth tides do not play a significant role in the deceleration of the Earth.

It is difficult to say to what extent the computed phase lag values might be in accordance with observational results, since the determination of instrumental lag is rather uncertain. The instrumental lag of recording apparatuses can be computed mathematically or it can be determined experimentally using some artificial gravity oscillation. But in both cases an insufficient knowledge of parameters and physical characteristics of the instrument makes the exact determination of the accurate instrumental lag rather doubtful.

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Age differences between Archaean cratons of eastern and southern Africa

ARCHAean greenstone belts have commonly been correlated on the basis of similarities in their stratigraphic successions¹. Conclusive radiometric tests of these correlations have rarely been made, because of the difficulty of dating the rocks of the greenstone belts themselves, and because of uncertainties in their relationships to the more easily dated granitoid rocks with which they are normally associated. We report here radiometric evidence from eastern Africa, which clearly contradicts previous lithostratigraphic correlations between the Tanzanian and Kaapvaal Cratons by establishing a minimum age difference between the uppermost parts of their respective greenstone belt successions.

In Table 1 the stratigraphy of the greenstone belts of the Tanzanian Craton (see Fig. 1) is compared with those of the Rhodesian and Kaapvaal cratons. The Kavirondian and Nyanzian systems, confined to the northern part of the Tanzanian Craton, are particularly well exposed near the Tanzanian–Kenya border towards Lake Victoria. There is no direct evidence that the Dodoman is older than the Nyanzian², only the presence of metamorphosed ultrabasic rocks in its type area³ supports the analogy with the Sebakwian and Lower Onverwacht suggested in Table 1.

The Kavirondian and Nyanzian systems were shown by Cahen and Snelling⁴ to be older than about 2,550 Myr. Subsequently, Old and Rex⁵ published a 3,100 Myr Rb–Sr isochron age for the Masaba granite, south-eastern Uganda, which they considered a minimum age for the Nyanzian. Field relationships suggest that the granite may be post-Kavirondian⁶, which means that the greenstone belts in eastern Africa would be comparable

Table 1 Radial variation of dissipation function Q

Depth (km)	Q
0–38	450
38–300	100
300–1,000	300
1,000–2,898	1,000

Table 1 Comparison of some African greenstone belts*

Tanzanian Craton	Rhodesian Craton	Kaapvaal Craton	Lithology
Kavirondian System (Unconformity)	Shamvaian System (Unconformity)	Moodies Group (Unconformity)	Sedimentary Group: coarse grits, conglomerates and shales.
		Fig Tree Group	Turbidites
Nyanzian System	Bulawayan System	Upper Onverwacht Group	Greenstone Group: mafic and salic flows and tuffs, banded ironstones and cherts.
Dodoman System	Sebakwian System	Lower Onverwacht Group	Ultramafic Group: dunites, peridotites, tholeiitic basalts, minor sediments.

*Modified after Anhaeusser *et al.*¹.

in age to the very ancient rocks of the Swaziland System⁷⁻¹⁰. (All Rb-Sr ages are calculated here with $\lambda_{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$, to facilitate comparison with published data on southern Africa.)

We have dated pre and post-Kavirondian granites in western Kenya by the whole-rock Rb-Sr isochron method. We have also reanalysed the samples of Masaba Granite studied by Old and Rex⁵, and have obtained very different results from those authors. Sample localities are shown on Fig. 2.

Two distinct phases of magmatism bracket the Kavirondian system. The Mumias Granite cuts post-Kavirondian structures in the Kakamega region, and has produced a distinct thermal aureole in the Kavirondian sediments to the north. This granite is contiguous with the Kitosh Batholith of Kenya, which becomes the Buteba Granite in Uganda⁵. The Migori G2 granite, the only body for which there is good evidence for a pre-Kavirondian age, intrudes the Nyanzian but is nowhere seen in contact with the Kavirondian. Its pre-Kavirondian status¹¹ is based on its petrographic similarity to some of the well-rounded boulders contained within a basal Kavirondian conglomerate

overlying the Nyanzian a few kilometres north-east of the G2-Nyanzian contact (Fig. 2). The Masaba granite has an uncertain relationship with the Nyanzian and Kavirondian systems, but its eastern boundary seems to truncate Nyanzian-Kavirondian contacts⁶.

Although the samples of Migori G2 are petrologically more varied than the very uniform boulders from the Kavirondian conglomerate, KEN 167 and 168 are porphyritic quartz monzonites which are indistinguishable in thin section from the boulders. The post-Kavirondian granites, also quartz monzonites, contain abundant biotite or chlorite, and rare hornblende, whereas the pre-Kavirondian samples are characterised by abundant hornblende.

Analyses were performed at the Universities of Leeds (Migori G2, Kavirondian boulders, and reanalyses of Masaba), Texas (MA 100-103, analysed by S. DeLong) Toronto and Carleton University. Concentrations of Rb and Sr were determined by isotopic dilution at Leeds and by X-ray fluorescence elsewhere, with an estimated precision of $\pm 1.5\%$ (1σ). Strontium isotope ratios have a precision of $\pm 0.03\%$ on critical samples (Migori G2), and 0.4-0.05% on the remainder. Numerous inter-laboratory comparisons showed no systematic differences.

Fig. 1 Geological setting of Tanzanian Shield. (Inset map after T. N. Clifford.)

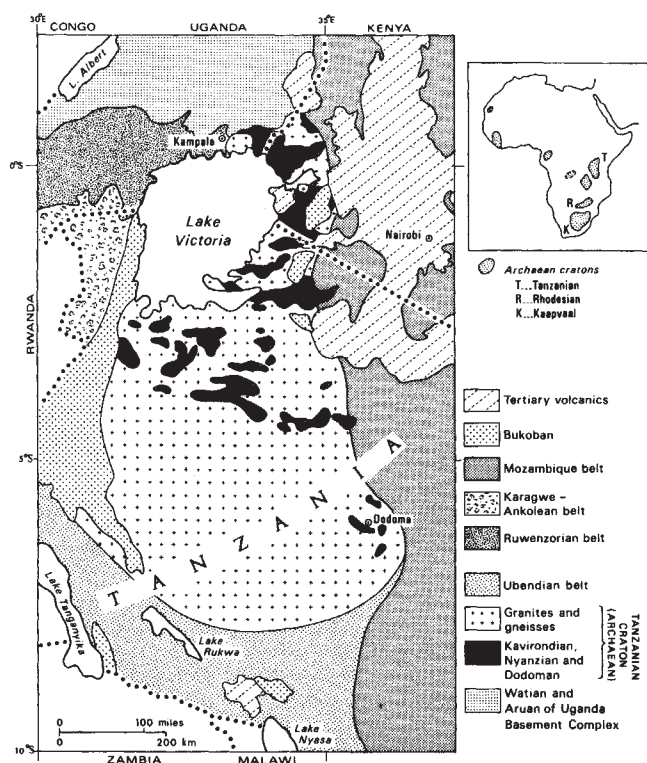


Fig. 2 Sample localities: Ma, Masaba Granite; Bu, Buteba Granite; Mu, Mumias Granite; Mi, Migori G2; Kc, Kavirondian conglomerate; T, Tertiary volcanics. Small Kavirondian outcrops are omitted.

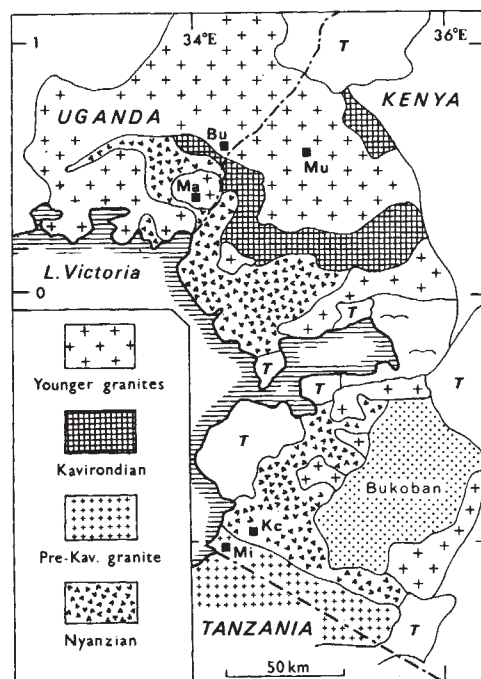


Table 2 Rb-Sr data from granites in north-western part of Tanzanian Craton

	Sample no.	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}$ (atomic)	$^{87}\text{Sr}/^{86}\text{Sr}$ (atomic)
G2 Migori Gold Belt SW Kenya	KEN 167	115	807	0.414	0.7181
	KEN 168	123	704	0.508	0.7210
	KEN 169	114	726	0.455	0.7196
	KEN 170	95	839	0.329	0.7143
	KEN 171	110	565	0.570	0.7239
	KEN 172	98	837	0.338	0.7146
	KEN 173	126	848	0.430	0.7186
Boulders, Kavirondian conglomerate	KEN 149	101	878	0.335	0.7153
	KEN 150	120	705	0.495	0.7213
	KEN 151	112	821	0.396	0.7183
	KEN 152	106	850	0.360	0.7171
	KEN 153	139	642	0.630	0.7246
	KEN 155	136	629	0.629	0.7283
Masaba SE Uganda	RO 400	64.5	121.3	1.542	0.7539
	RO 401	76.9	105.1	2.120	0.7810
	RO 402	63.1	36.1	5.07	0.8727
	RO 404	38.0	236	0.463	0.7166
	RO 423	18.4	222	0.240	0.7131
	MA 100	24	281	0.24	0.7116
	MA 101	33	231	0.41	0.7158
Mumias, SW Kenya	MA 102	70	151	1.34	0.7513
	MA 103	78	113	2.02	0.7727
	KEN 113	223	134	4.89	0.8831
	KEN 114	194	123	4.65	0.8731
	KEN 116	306	281	3.19	0.8077
Buteba, SE Uganda	KEN 117	186	614	0.87	0.7320
	KEN 118	196	631	0.90	0.7339
	*RO 405	272	96.9	8.35	0.9954
	RO 406	287	103	8.29	0.9976
	RO 407	233	112	6.15	0.9177

* $^{87}\text{Sr}/^{86}\text{Sr}$ determined at Leeds, Rb and Sr at Carleton.

Analytical data are presented in Table 2 and Figs 3 and 4. The post-Kavirondian samples, including Masaba, give ages of about 2,500 Myr (Fig. 3). The main differences between our data on the Masaba granite and those of Old and Rex⁵ lie in the Sr isotope ratios, which they apparently overestimated systematically. Our new analyses on the original Masaba samples (pre-fixed RO) are consistent with the results obtained by De-Long on MA 100–103. We consider the latter to be the more reliable; the samples were collected specifically for geochronological analysis, and they yield an isochron age of $2,500 \pm 80$ Myr, with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ (R_i) ratio of 0.7024 ± 0.0013 . The other post-Kavirondian granite, Mumias-Buteba, yields an isochron age of $2,530 \pm 50$ Myr ($R_i = 0.701 \pm 0.003$) consistent with the original data⁵ for these samples. Deviations from linearity in both isochrons suggest a measure of geological error, and the uncertainties quoted are estimates based on McIntyre's models 2–4 and York's model 1 (ref. 12).

Our minimum age for the Kavirondian system, $2,530 \pm 50$ Myr, is similar to that given previously⁴. A maximum age is obtained from the Rb–Sr isochron on Migori G2 (Fig. 4), which gives $2,800 \pm 120$ Myr ($R_i = 0.7013 \pm 0.0007$) with no significant geological error. The boulders do not yield an isochron, but they plot sufficiently near to the data on G2 to support the hypothesis that they were derived from that body; the discrepancies can reasonably be ascribed to the effects of weathering. There are no strong reasons to suspect the isochron age of G2 to be other than the date of emplacement.

The Kaapvaal counterpart of the Kavirondian System, the Moodies Group, is intruded by the Kaap Valley Granite, dated at $3,310 \pm 40$ Myr by the U–Pb method⁹. A possible older limit for the Moodies Group, $3,500 \pm 200$ Myr, is given by an Rb–Sr mineral isochron¹⁰ on a basaltic komatiite from the underlying Onverwacht group. Thus, there is an age difference in the range 500 ± 100 – $1,000 \pm 200$ Myr (a length of time comparable with the whole Phanerozoic), between the Moodies Group and the Kavirondian System.

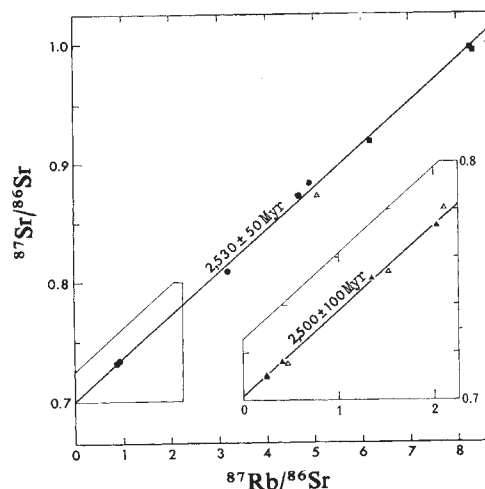
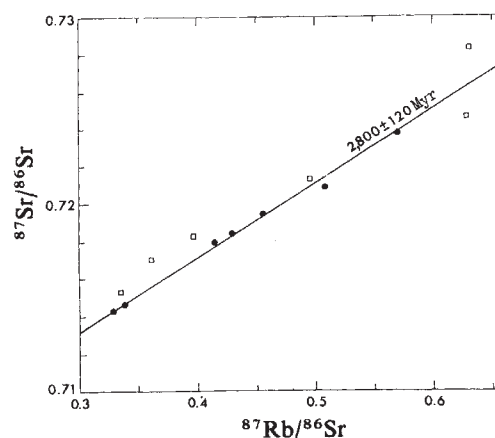


Fig. 3 Rb–Sr isochrons for post-Kavirondian granites. ●, Mumias; ■, Buteba; triangles, Masaba (closed symbols used for isochrons). Isochron calculations were made using the method of McIntyre and York¹². Uncertainties are 1σ (67% confidence). Mean square of weighted deviates (MSWD) = 7.2 (Mumias) and 5.5 (Masaba).

No granites older than Migori G2 ($2,800 \pm 120$ Myr) have been found in the Tanzanian Craton^{13–15}, and nothing unequivocally younger than $2,500 \pm 100$ Myr. In the Kaapvaal Craton the granitic rocks were emplaced over a much longer period, and are divided into two suites on geochemical grounds¹⁶. Granites of the older suite, dated at 3,400–3,000 Myr, can perhaps be considered to be geochemically analogous to the granites of the Tanzanian Craton. The 'younger plutons', all of which postdate the greenstone belts, have ages of 2,800–2,500 Myr, but possess no obvious analogues in eastern Africa in the appropriate age range (1,900–2,200 Myr). Further radiometric and geochemical measurements on the granites of the Tanzanian Craton are in progress, and will allow a more precise comparison between the Archaean histories of eastern and southern Africa.

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Fig. 4 Rb–Sr isochron for pre-Kavirondian granite from Migori, Kenya. ●, *In situ* samples; □, boulders. MSWD = 0.8.



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Numerical test of Palásti's conjecture on two-dimensional random packing density

RANDOM packing and random space-filling problems have received frequent attention¹⁻⁵. In one dimension, this topic is usually called the car-parking problem⁶⁻¹⁰. Palásti¹¹ generalised the parking problem to two dimensions and conjectured that the random packing density in the two-dimensional case is equal to the square of that in the one-dimensional case. Here we provide a thorough check on her conjecture and confirm its validity.

The procedure of two-dimensional random packing in Palásti's sense is as follows. Consider a rectangle T_{xy} with vertices $(0,0)$, $(x,0)$, $(0,y)$, (x,y) where $x,y > 1$. Fill the rectangle at random by unit squares whose sides are parallel to the coordinate axes. The centre of filling squares is assumed to follow a uniform distribution over the unfilled portion of the rectangle. If a unit square overlaps any of the previously placed squares or intersects with the boundary of T_{xy} , then it is discarded. The process continues until the remaining space is inadequate for a unit square. Let $M(x,y)$ denote the total

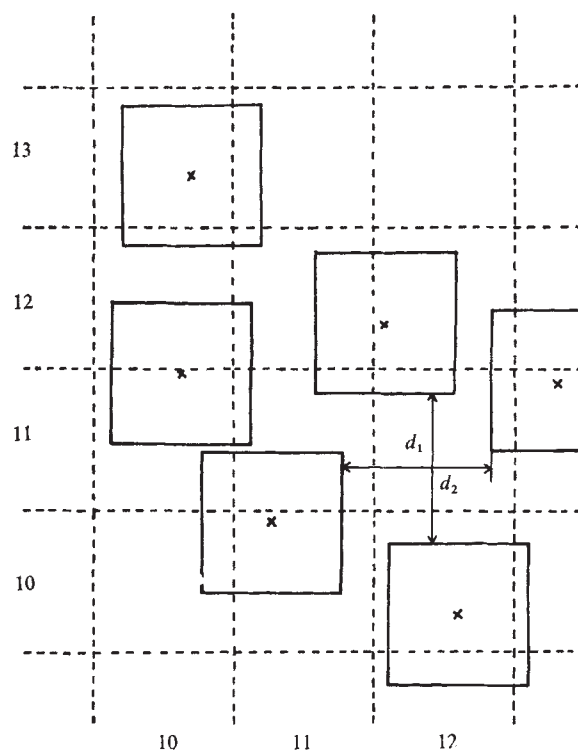


Fig. 1 Diagram illustrating the filling procedure. Dotted meshes represent unit square sections into which T_{xy} is subdivided. Unit squares whose sides are solid lines express cars. For example, the sections (10,13) and (11,11) belong to type I and type II, respectively. The section (12,11) is of type III since d_1 and d_2 are greater than unity. When (12,11) experiences 150 discardings of cars and still remains unfilled, we classify it in type III*.

number of unit squares placed in T_{xy} and $m(x,y)$ indicate the expectation of the random variable $M(x,y)$. Palásti¹¹ proved that there exists

$$\lim_{x \rightarrow \infty, y \rightarrow \infty} m(x,y)/xy$$

and conjectured that the limit is equal to $c^2 \approx 0.5589$ where

$$c = \int_0^\infty \exp[-2 \int_0^t (1 - e^{-u})/u du] dt \approx 0.7476$$

This is just the limit of the mean random packing density in one dimension⁶. Furthermore, she made Monte Carlo experiments for the four cases of $xy = 5 \times 15, 10 \times 15, 15 \times 15, 20 \times 15$, which always produced a value of 0.56. Solomon¹² reported similar experiments with results ranging from 0.50 to 0.55. In both cases, however, the size of the rectangle T_{xy} seems too small to test Palásti's conjecture stringently. We have devised

Table 1 Computer simulation results for mean packing density

Rectangle T_{xy} (xy)	Present work				Previous work		
	Lower bound	Upper bound	Mean	s.d.	Palásti (ref. 11)	Solomon (ref. 12) Mean	s.d.
5×15	0.5240 (0.4920*)	0.5347 (0.5080*)	0.5347 (0.5080*)	0.0317 (0.0329*)	0.56†	0.50270	0.02089
10×15	0.5173	0.5327	0.5293	0.0222	0.56†	0.53933	0.02188
15×15	0.5280	0.5462	0.5422	0.0170	0.56†	0.53644	0.01027
20×15	0.5350	0.5450	0.5430	0.0147	0.56†	0.53700	0.01261
20×30	0.5343	0.5495	0.5465	0.0073	—	0.54833†	—
40×40	0.5415	0.5574	0.5536	0.0040	—	—	—
60×60	0.5451	0.5608	0.5571	0.0043	—	—	—
100×100	0.5479	0.5625	0.5593	0.0018	—	—	—

* Another series of ten trials.

† Only one trial.

a computer program by which a Monte Carlo simulation of this problem can be made even for relatively large T_{xy} .

For simplicity we restrict ourselves to the rectangle T_{xy} with x and y integers. We subdivide T_{xy} into unit square sections whose number is xy . To avoid confusion between a unit square section and a filling unit square, we shall hereafter call the former a 'section' and the latter a 'car'. Instead of picking a centre point of a car at random in T_{xy} , choose a section with equal probability and then choose a centre of a car in that section. During the course of packing, we classify sections into three categories (Fig. 1). A section of type I contains a centre point of a car already placed. It is obvious that sections of this sort cannot be occupied by centres of cars again. A section of type II is defined as an empty section to which no centres of cars can be assigned. In other words, the arrangement of cars inserted in the neighbouring sections prevents the type II section from including a centre point of a car. Sections that belong neither to type I nor to type II are termed type III sections. At the first stage, all sections are of type III. As the procedure goes on, however, some of them change to type I or type II sections. Type III sections left unchanged still experience repeated discardings of cars. Those sections which have encountered 150 discardings of cars are excluded from the group of type III and renamed type III* sections. The iteration proceeds until no sections of type III remain in T_{xy} . The number of type I sections gives a lower bound of $M(x, y)$, and the total number of type I and type III* sections yields an upper bound. The difference of these bounds was about 1.5%. Furthermore, careful examination of type III* sections enables us to evaluate $M(x, y)$ with an error of at most 0.1%.

Table 1 shows the results of our computer simulations together with the data of Palásti and Solomon. Sample means and standard deviations over ten trials are listed there. Our results seem to be in better agreement with those of Solomon than with those of Palásti. As the size of T_{xy} increases, the mean packing density approaches $c^2 \approx 0.5589$. When $xy = 100 \times 100$, this value is estimated as 0.5593, which is very close to c^2 . Assuming that $M(x, y)$ is asymptotically normally distributed as in the one-dimensional case^{9,10}, we have a 95% confidence interval of (0.5593 ± 0.0013) . The validity of Palásti's conjecture is thus confirmed numerically.

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Detonation of fuel coolant explosions

IN certain circumstances the mixing of a hot liquid and a cooler, vaporisable one leads to an explosive rate of vapour production. Such explosions have occurred in foundries when molten metals and water mix¹, and when liquid natural gas is spilt on to water²; they may also occur in liquid-cooled nuclear reactors under accident conditions. It seems likely^{3,4} that in these events an initial disturbance causes motions which fragment some of the material and so allow rapid heat transfer; this produces

explosive expansion and further fragmentation, and so the reaction propagates through the medium. We give here a simple one-dimensional model of a system in which the two liquids are initially coarsely mixed, and show that there is the possibility of an extremely violent thermal explosion, the structure of which is analogous to that of a detonating chemical explosion.

Consider a strong shock front progressing steadily through the coarsely mixed material (Fig. 1); we assume (and justify below) that close to the front the flow velocities are sufficient to cause fine fragmentation of the hot material and rapid heat transfer. The front leaves behind a mixture which attains thermal equilibrium at high pressure; in so doing the material expands and drives the front forward.

Applying the equations of conservation of mass, momentum and energy to the material entering and leaving the front⁵ we deduce that the possible states (U_2, P_2, V_2) of the material leaving the front are related to the pressure P_1 , specific volume V_1 and internal energy U_1 of the material entering the front by

$$\frac{1}{2}(P_1 + P_2)(V_1 - V_2) = U_2 - U_1$$

This, together with the equation of state for the material leaving the front $U_2(P_2, V_2)$, defines a unique relationship between the possible values of P_2 and V_2 (called the Hugoniot or shock adiabat).

From the theory of detonation⁵ it can be shown that for a specific set of initial conditions, there is only one state (point O in Fig. 2a), for the material behind the front which ensures that the explosion is stable. In this state the velocity of the material leaving the front is just sonic with respect to the front—this is called the Chapman–Jouguet (C–J) condition. Sonic choking at the C–J plane enables the explosion front to propagate independently of the motion in the region behind it.

The explosion propagates with a velocity which is greater than the speed of sound in the medium ahead of the front. The pressure and density both rise at the shock front in the 'von Neumann spike' (point N in Fig. 2a), and the velocity in the frame of the front falls from the shock velocity to a low value (Fig. 1). Behind the shock, as fragmentation and energy transfer occur, the velocity increases, reaching the local speed of sound at the C–J plane. Pressure and density both fall in this region, along the straight line of constant mass flux NO in Fig. 2a.

Behind the C–J plane the material expansion continues. The form of the solution in this region is 'self similar'; that is the pressure and velocity profiles are always the same shape but their scale increases linearly with time. Thus the expansion rates in this region fall inversely with time, and for times much longer than the heat transfer timescale equilibrium will be maintained throughout, and the expansion will be adiabatic.

By analogy with the terminology for chemical explosions, we call a thermal interaction which propagates stably behind a shock front a 'detonating thermal explosion'. Because the explosion must satisfy the C–J condition, we can predict the propagation velocities and pressures without a detailed knowledge of the fragmentation and energy transfer processes, assuming only that energy transfer is complete at the plane a₁ which the C–J condition is satisfied (see, for example, ref. 6). Figure 2b shows the shock adiabat for a system consisting of equal volumes of tin, water and steam. The tangent from the initial point, $P_1 = 1$ bar, $V_1 = 0.37$ g cm⁻³, meets the curve at a C–J point of pressure ~ 1 kbar. The propagation velocity

$$v_1 = V_1 \sqrt{[(P_2 - P_1)/(V_1 - V_2)]} \sim 3 \times 10^4 \text{ cm s}^{-1}$$

If there is little or no vapour initially present the Hugoniot curve remains more or less unchanged, but the point from which the tangent is drawn moves to a lower specific volume so that the pressure of the C–J state increases considerably. The propagation velocity and the velocity differential causing fragmentation are also increased by the absence of vapour.

The model as so far outlined does not demonstrate that a detonation will occur, merely the conditions which it will fulfil if it does. To demonstrate the realisability of such an explosion we must consider the fragmentation processes behind the front.

In a detonating chemical explosion, the leading edge of the reaction front is a shock in the unreacted material; the compression raises the temperature of the material passing through it to such a level that combustion occurs close behind the shock. We consider that the structure of a detonating thermal explosion may be similar; a simplified picture (Fig. 1) is that the leading edge of the reaction region is a shock in which the vapour blankets are collapsed causing some degree of mixing and allowing efficient heat transfer to begin. The steep pressure gradient at the shock front accelerates the materials entering it at rates dependent on their densities, so causing velocity differentials between the residual vapour, coolant and fuel. If these velocity differentials are sufficiently large, fragmentation will occur before velocity equilibrium is re-established. Such mechanisms for the breakup of drops have been observed in chemical detonation of two-phase systems where the fuel is in droplet form in a gaseous oxidising atmosphere⁷.

We may use the data of Simpkins and Bales⁸ on the breakup of liquid drops behind shock fronts to examine this event in more detail. They showed that a drop of radius r_0 , density ρ' in a flow of velocity u , and density ρ would break up in a time t given by

$$(\rho/\rho')^{1/2}ut/r_0 \simeq 44B_0^{-1/4}$$

Here B_0 is the Bond number, given by

$$B_0 = (\rho'gr^2)/\sigma = \frac{3}{8}C_DWe = (3u^2r_0C_D)/8\sigma$$

where C_D is an effective drag coefficient (~ 2) and σ is the surface tension of the drop. We compare the breakup time t with that required to achieve velocity equilibrium, namely

$$t' \simeq (8/3)(\rho'r_0)/(\rho u C_D)$$

The breakup time must be shorter than this for successful

Fig. 1 Geometry and schematic pressure and velocity profiles of a one-dimensional explosion.

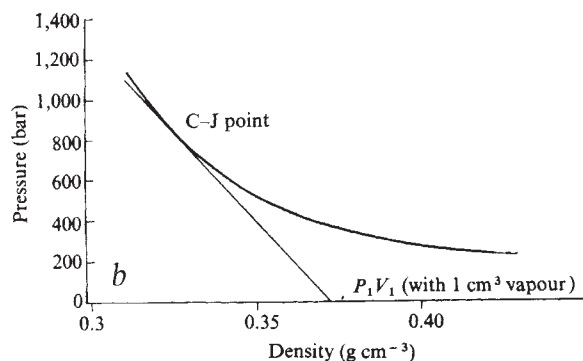
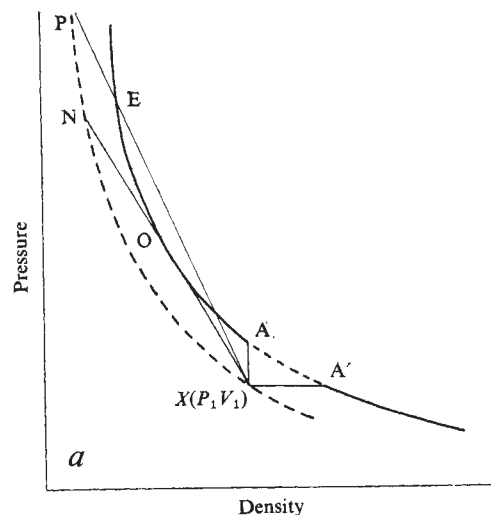
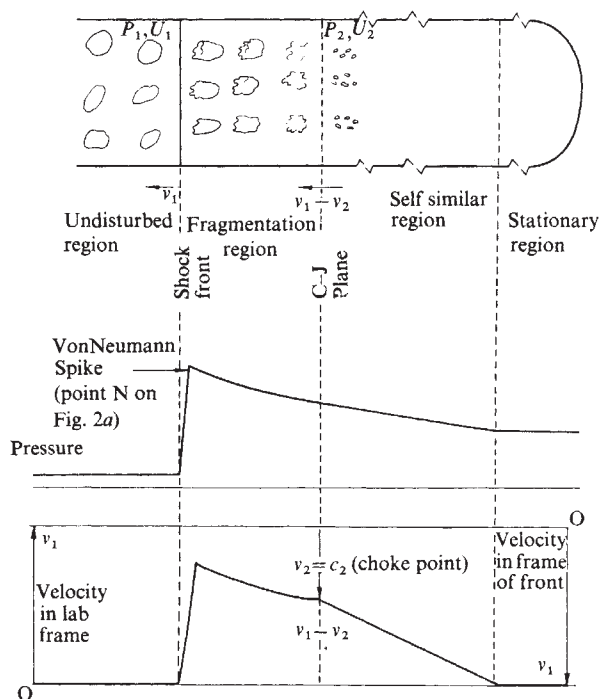


Fig. 2 a, Schematic shock adiabat. Solid curve, reacted material; dashed curve, unreacted material. b, Shock adiabat for an initial mixture of equal volumes of tin at 1,000 °C, water at 100 °C and steam.

fragmentation. For tin drops of 1 cm diameter in water this condition is satisfied if the Bond number is greater than $\sim 10^4$. The differential velocity, u , is initially greater than $v_1 - v_2$ (Fig. 1), where $v_1 - v_2 = \sqrt{[(P_2 - P_1)(V_1 - V_2)]} \sim 10^4 \text{ cm s}^{-1}$ (Fig. 2b). Thus Bond numbers are greater than 10^8 , and breakup by Taylor instability is achieved in typically $\sim 2 \times 10^{-4} \text{ s}$, $\sim 10 \text{ cm}$ behind the front. The liquid drop will break into particles a micrometre or so across ($\rho u^2 r / \sigma \sim 8$), and so the time for heat transfer will be much shorter than that for breakup. Thus, a short distance behind the front energy transfer will be complete and a homogeneous equilibrium state will be produced. These equations, though derived for motion down a long tube, should apply in any explosion if the reaction front is plane on a scale of many times its thickness so that all flow velocities are normal to it. Single-phase chemical detonations have reaction fronts which are only a few tenths of a millimetre wide so that detonations can occur even in very small regions without external constraint. On the other hand, two-phase detonations, whether chemical or thermal, have relatively long reaction lengths. True one-dimensional detonation can only occur in constrained geometries, or regions of coarse intermixing which are large compared with the reaction length.

We show now that the experiments so far performed in which large efficiencies have been achieved have approached the conditions necessary for detonating thermal explosions.

Consistently high pressures (up to 600 bar) have been observed when columns of water are impacted into molten materials in strong tubes⁹. It has been observed that material is excavated to a depth of about one tube width by each successive bounce of the column, and that the largest pressure peak is usually produced on the second impact. If material which was

excavated on the first bounce is coarsely distributed over a few tube diameters at the bottom of the column, then the pressure pulse provided on second impact may trigger a detonating thermal explosion in this rather short (~ 5 cm) but roughly homogeneous and constrained medium. Experiments in which longer initial distributions of material were produced would provide a good test of the model.

We have observed explosions of quite high efficiency between Freon-22 and hot water¹⁰; on a foundry scale efficient interactions in metal-water systems have also been reported. In both cases there is photographic evidence for a degree of intermixing of the two components before explosion, though it is probable that in neither case is the characteristic dimension of the system much larger than the reaction length. Such explosions are thus intermediate in character between those in very constrained geometries and those in unconstrained stratified media.

Experiments in horizontally stratified and unconstrained metal-water mixtures have provided direct evidence for explosion propagation⁴. The reaction region is observed to propagate at only moderate velocity ($\sim 3 \times 10^3$ cm s⁻¹) along the fuel-coolant interface, and pressures of a few tens of bars are generated. These interactions do not fulfil the conditions for a one-dimensional detonating explosion; there is little premixing of tin and water, and the reaction front is necessarily of a size comparable with its width, so that sideways flow out of the front is very important. Accordingly, vapour generation is not suppressed, and the speed of sound in the front will be low. But it seems possible that for reasons of stability similar to those applying to a one-dimensional detonating explosion, the Chapman-Jouguet condition also holds for these two-dimensional explosions. Thus, because of the low speed of sound at the front, material will leave the front rather slowly and so the propagation itself will be slow; the low propagation velocities, and hence low efficiencies, seen in our small-scale experiments may also be characteristic of larger-scale explosions in unconstrained stratified media. But the first slow passage of the reaction front over a pool of metal may not cool all the available metal and could leave behind a coarsely mixed region through which a second, more efficient, explosion could propagate; this is indeed consistent with the photographic evidence of our larger tin-water interactions.

Although further work is necessary for a full quantitative theory of two-dimensional explosions, the present one-dimensional model is applicable to large scale events in, say, nuclear reactors. The SPERT 1D reactor was destroyed by an explosion which occurred when one third of the fuel was melted in an overpower transient. The high fraction of vapour in the core ($\sim 30\%$) at the time of the explosion presents difficulties for most explanations of the large pressures generated (~ 250 bar), see ref. 11), but these are easily understood on our model.

Fuel melting could also arise in fast reactors under fault conditions, and our calculations¹² show that pressures of the order of 15 kbar could be produced from large scale events involving sodium and molten uranium dioxide.

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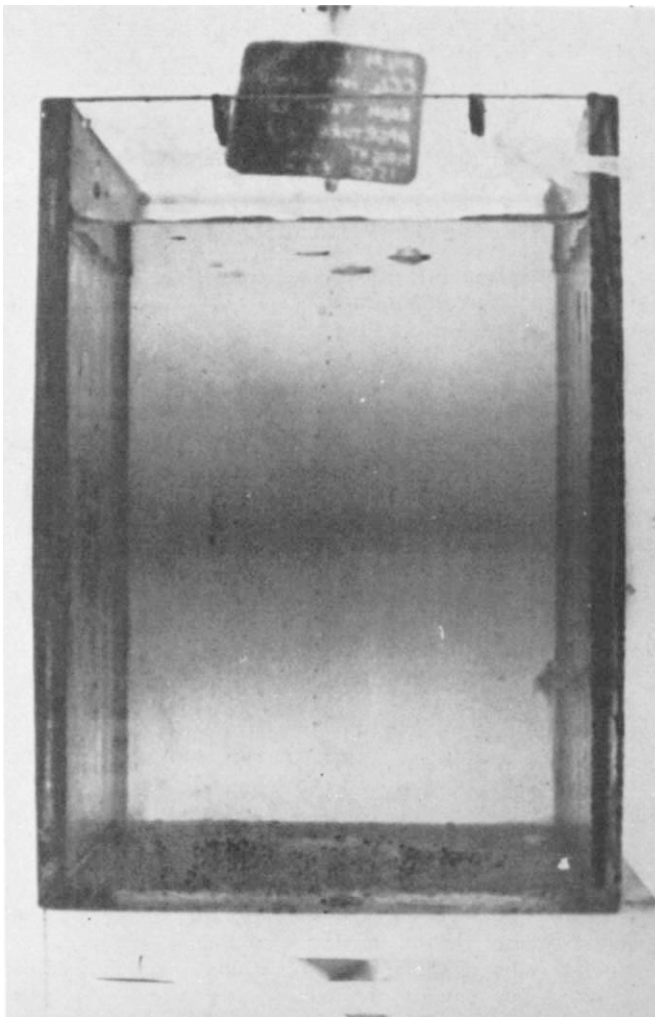
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Fall of liquid metal into water

THERE is a possibility of explosions under a wide variety of conditions¹⁻⁶ when liquids are rapidly mixed. When molten tin is poured from a crucible into water in defined conditions, a rapid explosive interaction takes place producing a sponge-like mass of debris⁷⁻⁹. We have repeated these experiments using an apparatus which provides small (~ 0.25 g) and reproducible single drops of molten tin, and have been able to determine quantitatively the dynamics of the interaction by a photographic technique. Using a metal temperature of ~ 570 K and water at room temperature metal drops were produced which entered the water in a straight vertical line but were then observed to deviate suddenly and sharply from their course without losing their pear-like shape. This metal temperature was just below that necessary to produce explosive interactions for the given water temperature.

We have photographed the fall of drops of molten tin at 570 K, and mercury and carbon tetrachloride at room temperature, into tap water at room temperature, using stroboscopic illumination. The path of a falling drop is clearly visible, both in the air and below the water, and the velocity may be determined from the image spacing. Although mercury and carbon tetrachloride fall in vertical straight lines to the first order (Fig. 1), molten tin drops

Fig. 1 A carbon tetrachloride drop falling from a height of 2 cm into water. Both liquids were at 293 K, and the drop was photographed in a stroboscopic light at 1,500 flashes min⁻¹. No significant deviation can be seen.



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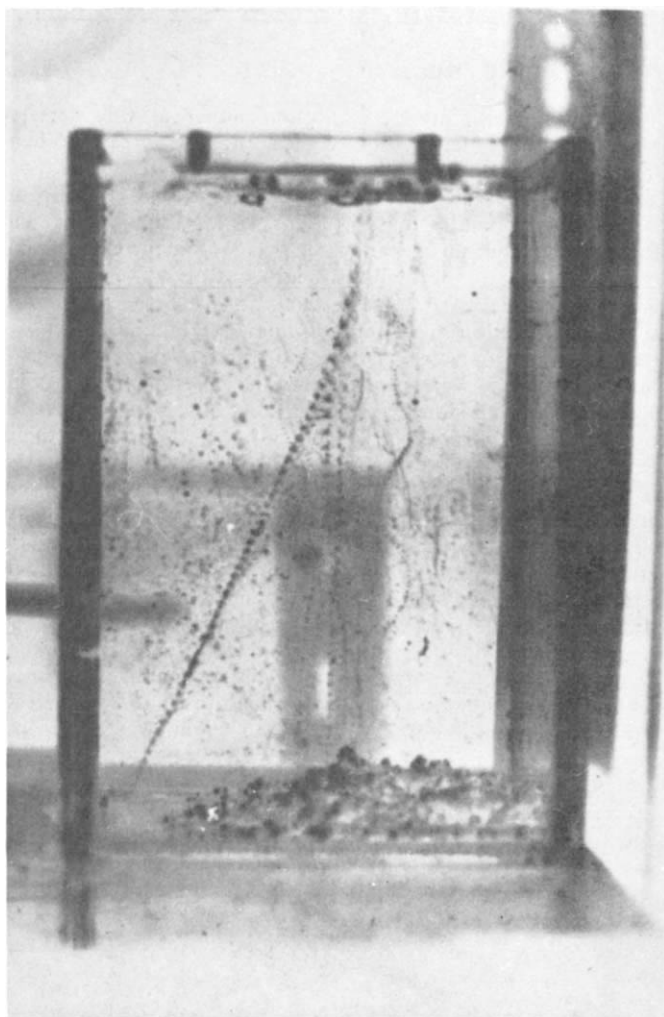


Fig. 2 Molten tin drops at 570 K falling from a height of 23 cm into distilled boiled water at 293 K. The drops were photographed in a stroboscopic light at 9,500 flashes min^{-1} . A significant deviation can be seen.

(Fig. 2) initially fall in vertical straight lines, but after a few centimetres in the water deviate from this straight line by up to $\frac{1}{2}$ rad.

It has been suggested⁷⁻⁹ that fragmentation of the molten tin is linked with the collapse of a vapour blanket surrounding the molten metal during the change from stable film boiling to transition boiling. Using a model for the collapse of a vapour bubble developed by Plesset and Chapman¹⁰, Buchanan and Dullforce⁷ predict the velocity and dimensions of a slug of water resulting from the collapse of a bubble of initial radius R_m , which then penetrates the liquid metal initiating the first cycle of a fragmentation process. From our photographs, using simple particle dynamics, it is possible to find the direction and magnitude of momentum imparted to the tin drop. Assuming that this momentum results from the collapse of a single vapour bubble adjacent to the tin drop, R_m can be evaluated. Values of the momentum imparted lay between 4 and 12 g cm s^{-1} , and the momentum was directed upwards at angles to the vertical between 0.87 and 1.22 rad. Corresponding values of R_m were between 0.25 and 0.35 cm. These seem too large for single bubble collapse to be a complete explanation for an initiation of the fragmentation process, at least for molten tin drops of radius 0.15 cm.

Work has been carried out¹¹ on quenching hot, solid metal spheres by moving them at a constant speed (of a similar value to that of our tin drops) through a tank of water. A phenomenon termed a "transplosion" is described,

which takes place between stable film boiling and transition boiling. In less than 0.25 ms the vapour shell is explosively destroyed giving rise to a regime of boiling described as pulsation boiling, which consists of a thin oscillation vapour film. This then progressively changes to nucleate boiling from the front of the sphere to the back, with a pulsating vapour ring separating the nucleate boiling zone from the remaining thin vapour film. If the transplosions or the pulsating vapour rings occur in a similar fashion on molten tin drops, they could perhaps provide an explanation for the considerable momentum found to be imparted during the quenching process.

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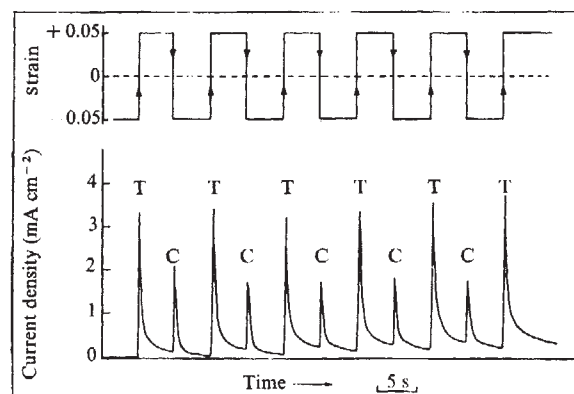
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Strain enhanced dissolution effects in the corrosion-fatigue failure of metals

It has been suggested¹ that yield assisted dissolution, which occurs during the corrosion fatigue of high carbon steels in aqueous sodium chloride solutions, may be responsible for the initiation of cracking. Furthermore, it is likely¹ that at low stresses the process of crack initiation largely determines the overall corrosion-fatigue life. It now seems that these significant dissolution effects can be produced and observed in metals subjected to strain cycling in corrosive solutions.

We have studied the anodic dissolution of mild steel, aluminium and stainless steel, during cyclic plastic deformation in aqueous electrolytes, in order to simulate on a macroscopic scale the local conditions that occur at stress concentrations during corrosion fatigue. Specimens held under potentiostatic

Fig. 1 Dissolution transients in 18/8 stainless steel, strain cycled in 3.7 M H_2SO_4 at 1,042 mV (normal hydrogen electrode—n.h.e.)



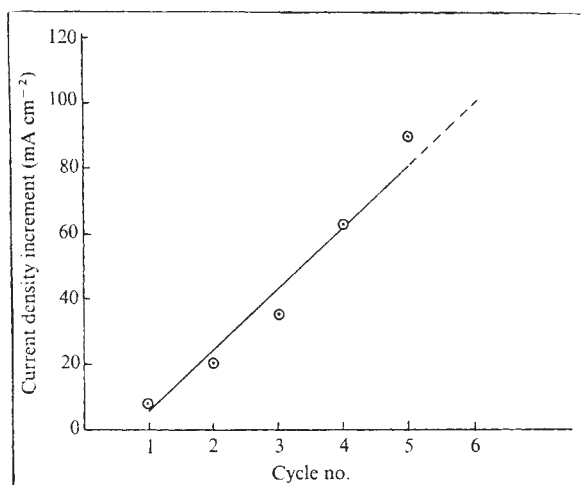


Fig. 2 The variation of dissolution transient magnitude with cycle number at a potential close to the active-passive transition, using 18/8 stainless steel in 3.7 M H_2SO_4 at 262 mV. Strain, -0.05 to $+0.05$.

control in the electrolyte were subjected to square wave strains in the range ± 0.02 to ± 0.05 at strain rates of $\sim 0.30 \text{ s}^{-1}$. These strain levels were chosen to provide a sufficient number of slip steps on the surface of the metal to give dissolution effects large enough to be measured. In practice, overall strains of this magnitude are unlikely to be encountered even at stress concentrations. Even when small local plastic strains occur, however, the emergent slip steps will behave chemically in a similar manner.

Dissolution transient currents were observed during both tensile and compressive strain reversals. The transients reached peak values during the application of the strain or at the position of maximum strain, and decayed during the constant strain portion of the strain cycle (Fig. 1). The magnitude of the transients, their position relative to the strain cycle and their decay characteristics have been found to depend on several factors, including the potential of the metal surface, the magnitude of the strain reversal, the strain rate and the tendency for surface films to form.

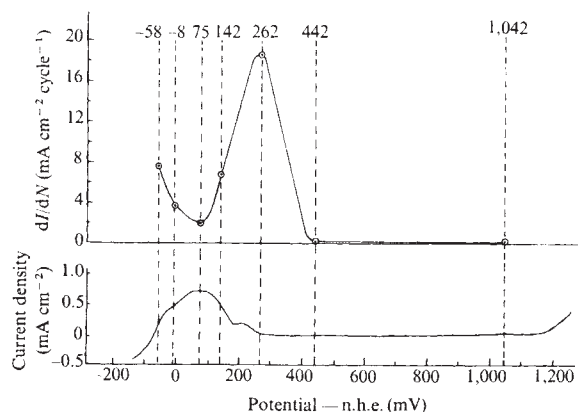


Fig. 3 The change of dI/dN relative to the anodic polarisation curve for 18/8 stainless steel in 3.7 M H_2SO_4 .

A particularly interesting type of behaviour was observed with 18Cr-8Ni (18/8) stainless steel in 3.7 M H_2SO_4 where repeated strain cycling at active and just passive potentials caused a progressive increase in the magnitude of the tensile dissolution transients. The initial transient at all potentials was found to be $< 10 \text{ mA cm}^{-2}$; at potentials close to the active-passive transition, however, the transients were found to grow by an order of magnitude

within six or seven strain cycles (Fig. 2). Assuming that dissolution was concentrated on emergent slip steps at the metal surface these transients represent a local increase of $> 1,000$ in the dissolution rate. The rate of increase of the peak value of the current transients per cycle, dI/dN , varies with potential for a cyclic strain of ± 0.05 (Fig. 3). It is evident that dI/dN reaches maximum values over a limited potential range coincident with the active-passive transition region; this effect has also been observed at strain levels below ± 0.05 . Dissolution effects occurring beyond 10 or 15 cycles have not been investigated because of experimental difficulties in continued cycling at high strain levels. It cannot, therefore, be assumed that dI/dN will remain constant during subsequent cycling.

If, in corrosion fatigue, stress concentration effects are sufficiently high to induce local plastic deformation on the surface of the metal then the observed dissolution behaviour is likely to occur and could assist crack initiation by a combination of reversed slip and dissolution². The evidence suggests that for stainless steel in H_2SO_4 the maximum contribution of dissolution to the initiation of cracking should occur at the active-passive transition. Corrosion-fatigue data on 18/8 stainless steel in H_2SO_4 at controlled potential is not available. It is interesting to note, however, that Spähn³ has found the minimum corrosion-fatigue properties of a 13% Cr stainless steel in H_2SO_4 occur at potentials in the active-passive range; that is, at potentials equivalent to those where we observe dI/dN to reach a maximum value.

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Tough fibrous composites

GORDON and Jeronimidis¹ and Atkins² have noted that increased fracture toughness in fibrous composites can be obtained by an enhanced decoupling of the fibre-matrix interface. Gordon¹ has pointed out that in the case of wood (natural cellulose), the decoupling of the 'reinforcing elements' from the rest of the structure is stress dependent and the cells in the vicinity of the fracture face can extend by about 20 per cent before breaking, absorbing a great deal of energy in the process. The failing strain of the material as a whole is, however, about 1%. Atkins² pointed out that increased works of fracture can be obtained in boron fibre epoxy resin composites by arranging for a distribution of strongly and weakly bonded interfacial regions along the fibres. During crack propagation the weakly bonded regions allow greater lengths of fractured fibres to be pulled out of the matrix with a concomitant increase in the work of fracture. The tensile strength of the composite system is not affected significantly but the overall failing strain is again limited by the failing strain of the reinforcing fibres to about 1%.

It has been pointed out^{3,4} that the fracture toughness of fibrous composites can be enhanced by a stress controlled decoupling mechanism and, moreover, that fibre fracture can thus be prevented. Very large tensile deformations are thus rendered possible as continuous reinforcing elements are pulled through the composite structure. The design and behaviour of experimental non-fracturing reinforcing elements has been reported^{5-7,12} and the enhanced work of fracture of sheet metal reinforced with such elements has been observed⁸⁻¹⁰.

Not only do such systems possess fail-safe characteristics, because of the non-fracturing nature of the reinforcing fibres, but the reinforcing phase is completely insensitive to stress concentrations. Because of this, these systems can absorb very much more mechanical energy without fracturing than the toughest work-hardening metal alloys in circumstances where the metal would suffer localised failure¹¹. That is, of course, the normal situation when sheet metal structures are subjected to localised loading by the impact of a missile or by any other means. Prototype systems based on the stress-controlled decoupling principle are under investigation as potential energy absorbing components of motor vehicles (R. S. Millman and M. J. Chappell, unpublished).

Studies have been made of the energetics of crack growth in metal plates loaded in tension under fixed grip conditions and reinforced by non-fracturing elements¹⁰. The fracture mechanics of these composite structural systems is quite different from those of other materials because the primary reinforcing phase is completely crack insensitive and also inhibits crack growth in the metal plate. Within a specified envelope of conditions, unstable crack growth in the metal plate is not energetically possible, whatever the length of the crack. Within these specified conditions the work of fracture of the composite system, calculated in the conventional way, becomes, in effect, infinitely large.

It is interesting to speculate whether analogous stress controlled decoupling mechanisms could be made to operate at the molecular level on the cross links between the molecular chains of polymeric systems.

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Organochlorine residues in Antarctic snow

DDT is a useful model compound for studying the circulation of a toxic pollutant in the global environment^{1,2}. An understanding of this process could in future be related to potentially more hazardous materials. Present models of the dynamics of DDT circulation can account for only a small fraction of the amounts of DDT and DDE which are known to have been released into the environment. Major unknowns include the extent to which the atmosphere and oceans act as reservoirs and the transfer rate of these residues from the atmosphere to the oceans where, according to present ideas, they may be removed from circulation by transfer to the abyss³. Such atmospheric and oceanic transport mechanisms may carry pollutants into the ecologically protected area of Antarctica and it is necessary to assess the extent to which this is occurring and the relative importance of alternative input routes. The atmosphere has been assumed to play the major role in the transport cycle but there is a lack of supporting data. We report here levels of DDT and metabolites

in Antarctic snow which suggest that the role of the atmosphere in the transport of DDT may have been overemphasised.

Measurements of global background levels are hampered by contamination problems at the very low concentrations found⁴, by the proximity of local sources of emission, and by interference from other more dominant organic components (as in oceanic measurements). Studies in the interior of Antarctica largely overcome the last two problems. Subzero temperatures throughout the year combined with negligible biological activity ensure minimal chemical alteration or diffusion of trapped pollutants after deposition of the snow. The year and season of deposition of a particular snow sample may be established by investigation of the stratigraphy of the snow section from which it was taken. The present study forms part of an attempt to establish upper limits of concentration of organochlorine residues in contemporary Antarctic precipitation.

Representative snow samples from the previous 5–10-yr accumulation were collected during December 1969 on a journey of 450 km inland from the British Antarctic Survey's Halley Bay station (75°31'S, 26°42'W). Sampling procedures were designed to reduce contamination to a minimum by repeatedly trimming large snow blocks before packing them for transport. The snow was returned to England in the frozen state, wrapped in pre-cleaned aluminium foil and sealed in steel containers.

Samples for analysis were obtained by continued trimming of the snow blocks in a glove box under purified nitrogen at -10°C . Analyses were carried out in a closed glass system¹³ under purified nitrogen. The system allowed melting, meltwater transfer, and extraction and concentration steps to be carried out in sequence so that at no stage did the samples contact a laboratory atmosphere. Continuous cyclic liquid–liquid extraction was used to transfer the organochlorine components in 1 l aliquots of meltwater into 20 ml hexane. The hexane solution was subsequently concentrated to 20 μl . Meltwater samples, to which had been added radiolabelled pp'DDT at the $1 \times 10^{-12} \text{ g g}^{-1}$ level, were used to check the efficiency ($>90\%$) of these procedures. Extracted components were identified without cleanup using a Pye 104 gas chromatograph with electron capture detection and $3 \text{ m} \times 3 \text{ mm}$ glass columns packed with 1.5% OV-17/QF-1 and 2% SE-30 on 100–200 mesh Chromosorb G. Identification of DDT was confirmed by treating the extract with alkali, which eliminated the DDT peak and enhanced the DDE peak. Detection limits, which were imposed by incidental contamination, were $3 \times 10^{-14} \text{ g g}^{-1}$ water for both DDT and DDE.

The data reported here were obtained from 10 snow samples representative of accumulation laid down between 1965 and 1969 at two sites, 360 and 400 km from the coast, where possible interference from oceanic components was minimal. Results from two duplicated samples agreed within experimental error (10% at $1 \times 10^{-13} \text{ g g}^{-1}$ to 50% at $2 \times 10^{-14} \text{ g g}^{-1}$). Peaks coincident with pp'DDT and pp'DDE occurred in all samples studied. The total concentration of DDT and its metabolites (DDT-R) ranged from 0.1 to $2.0 \times 10^{-12} \text{ g g}^{-1}$; however pp'DDE was consistently more abundant than pp'DDT by a factor of 1.3 to 1.8. There was no evidence for families of other components corresponding to polychlorobiphenyls (PCBs) in the 50–60% chlorine composition range, which are widespread in the northern oceanic areas⁵ and have been reported in birds which range north of the Antarctic Convergence⁶.

Our data contrast with earlier measurements⁷ made on snow from Plateau Station, Antarctica where some of the samples were reported to contain $40 \times 10^{-12} \text{ g g}^{-1}$ of pp'DDT alone. These figures are comparable with average measurements made in England over the same period⁸. It is evident that studies more widely based both in time and space are required in Antarctica.

Higher levels of DDE than DDT have also been reported in some English rainwater samples⁸, particularly outside the spraying season when DDT levels were relatively low. This could be explained simply by evaporation of the more volatile metabolite DDE from deposited DDT which has undergone microbial degradation. The increased DDE/DDT ratio indicated by the Antarctic samples could point to further photodegradation of

DDT vapour in sunlight⁹ during atmospheric transport. The very low background levels add further weight to Cramer's² suggestion that perhaps too much emphasis was placed on the atmosphere as a reservoir and transporter of DDT in an earlier model described by Woodwell¹ in which $60 \times 10^{-12} \text{ g g}^{-1}$ was considered to be a global average for precipitation. Further sources of chemical¹⁴ and biological degradation must be sought.

Previous studies^{6,10-12} of animal species which remain closely associated with the Antarctic continent throughout their life cycle (Emperor penguins, Adélie penguins, crabeater seals and snow petrels) have yielded lipid analyses of total DDT-R ranging from 0 to $200 \times 10^{-9} \text{ g g}^{-1}$. The average content of all samples so far studied is approximately $90 \times 10^{-9} \text{ g g}^{-1}$. Taking an initial value of $1 \times 10^{-12} \text{ g g}^{-1}$ DDT-R in Antarctic precipitation, an enrichment factor (concentration in animal/concentration in environment) of about 90,000 is obtained. This is compatible with values found elsewhere in the environment³. The implication is that the animal species which remain close to the Antarctic continent are in equilibrium with the surrounding air and natural water. It is not necessary to invoke a passage up the food chain of pesticide residues from oceanic nutrients drawn southwards across the Antarctic Convergence.

The extremely low levels of PCBs in our snow samples ($5 \times 10^{-14} \text{ g g}^{-1}$) agree with data⁶ from Adélie penguins and snow petrels, in which the ratio of DDT-R/PCBs is much greater than that found in species which range north of the Antarctic Convergence, despite the marked increase in DDT-R in these species^{6,12}. These results indicate that the Antarctic environment is much more strongly shielded from penetration by PCBs than by DDT-R. This would tend to argue against the idea that, at least on a global scale, the atmosphere is the predominant mode of transport for PCBs.

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Mutation affecting taste perception in *Drosophila melanogaster*

ANALYSIS of mutant organisms can elucidate by contrast the development of the normal phenotype and this approach can be used to break down behavioural characteristics into their embryological and physiological components. Taste in insects is a characteristic suitable for such a study. Genetic analysis of taste in *Drosophila melanogaster* can complement the electrophysiological and anatomical information on taste organs of flies obtained mainly from *Calliphora* and *Phormia*. The taste organs of flies are located predominantly on the labella at the tip of the proboscis, and on the tarsi. On each half labellum of *Drosophila* there are 36–42 taste bristles on the surface and some 25 taste pegs between the pseudotracheae. All but five or six bristles are penetrated by the dendrites of four chemoreceptors (the remaining ones having two dendrites), while there is only one chemoreceptor in each interpseudo-

tracheal peg. Each taste organ is also provided with a mechanoreceptor neurone (R. F., Bleiser-Avivi and J. A., in preparation). Of the four dendrites penetrating to the pore at the tip of the taste bristles in *Phormia*¹⁻³, one is a water sensor, one reacts to sugars and two react to salts⁴⁻⁶.

The normal fruit fly responds to stimulation of its taste organs by a vigorous extension (or retraction) of the proboscis which may be followed by consumption of the solution offered. A standard testing procedure was developed: 4–5-d-old flies were put into funnels covered with a nylon net; they were starved and desiccated for 3 h at 28 °C and then the funnels with the flies were transferred to Petri dishes containing a cotton wool pad soaked with a test solution to which a red food colouring was added. The flies were allowed to consume the solution through the net for 20 min (at 28 °C). The proportion of flies with red coloured intestines expressed the reaction of the flies to the taste of the solution. Wild type *Drosophila* consume a 0.1 M sucrose solution but reject it when 1 M NaCl is added.

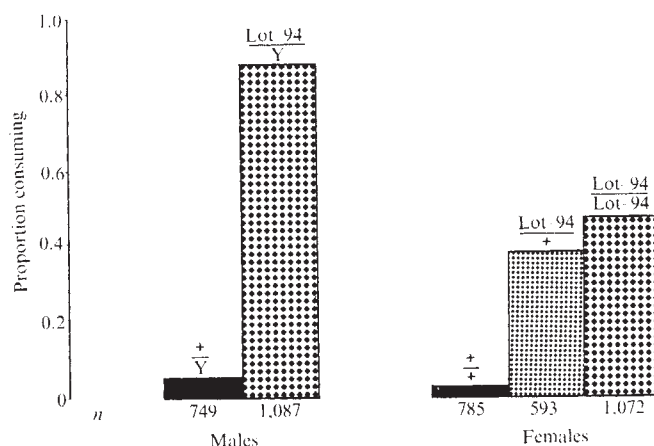


Fig. 1 Proportion of flies consuming the test solution (1 M NaCl+0.1 M sucrose) under standard conditions: 4–5-d-old flies, 3 h starvation and desiccation at 28 °C, 20 min feeding at 28 °C. +, The normal allele of the Lot-94 salt-tolerant mutation (in the heterozygous females it was carried over the In(1)FM6, $y^{31d} sc^8 dm B$ chromosome). n, Number of flies tested.

Mutations were induced by feeding young males of the wild-type Qiryat-Anavim (QA) stock with a 0.2% ethylmethane-sulphonate +1% sucrose solution for 24 h. The treated males were mated to yellow, attached-X females. F₁ progeny were scored for salt tasting with a standard screening solution of 1 M NaCl +0.1 M sucrose. About 3% of wild-type males consume this solution normally. All males with red intestines were mated individually to attached-X females and cultures in which a high proportion of the male progeny consumed the screening solution were maintained. The suspected X-chromosome mutations to tolerance to the taste of salt were designated 'Lot'. Three Lot mutations have been characterised so far⁸. One, Lot-94, was mapped to the left of *f*, at 55.5–56.0 on the X-chromosome (J. A. and Baker, in the press). Homozygous females of this mutation were less insensitive to NaCl solutions than the mutant males. This might be only partly the result of the generally greater reluctance of females to consume any solution under our experimental conditions. No clear conclusion on the dominance of Lot-94 could be reached, since in some heterozygous combinations it reacted like a partial-dominant mutation (Fig. 1), while in others it was completely recessive (see controls in Table 2). Another mutation, Lot-114, probably allelic to Lot-94, was completely recessive in all combinations tested. The third mutation, Lot-235, is a recessive mutation at another locus.

Response to labellar and tarsal stimulation was tested separately to find out whether the Lot-94 mutation affected both sites. QA and Lot-94 flies were fixed in rows on a double-

Table 1 Response of wild-type (QA) and mutant (Lot-94) males to 0.1 M sucrose (Suc) and to 1 M NaCl+0.1 M sucrose (Mix)

a, Proportion of flies extending proboscis when solution was applied up to three times									
Applied		QA		Lot-94					
to		Suc	Mix	Suc	Mix				
Labellum		0.91±0.08	0.69±0.08	0.91±0.11	0.77±0.19				
Tarsus		0.64±0.16	0.31±0.12	0.72±0.13	0.16±0.10				
Each proportion is based on 150–220 flies									
t-tests QA against Lot-94: Labellum, Suc $t = 0$; Mix $t = 0.97$. Tarsi, Suc $t = 1.14$;									
Mix $t = 2.90$, $P < 0.01$									
b, Duration (s) of consumption in flies that extended proboscis									
		QA				Lot-94			
		Suc	Mix	Suc	Mix				
Expt	No. of	Duration	No. of	Duration	No. of	Duration	No. of	Duration	No. of
	flies	(s)	flies	(s)	flies	(s)	flies	(s)	flies
1	14	> 30	16	4.8± 3.3	14	> 30	17	13.4± 8.0	
	3	18.7			3	24.0			
2	18	> 30	21	5.1±5.2	17	> 30	22	16.9±11.3	
	5	26.2			4	27.8			
3	25	> 30	25	5.2±4.7	20	> 30	30	16.8± 9.4	
	6	24.7			12	21.8			

Maximum feeding time was 30 s.

Table 2 Salt-tolerant gynandromorphic flies

Organ	Proportion of flies			Fate map distances*	
	Both sides non-mutant	One side non-mutant one side mutant	Both sides mutant	Af	ff'
Antenna	0.20	0.52	0.79	28.2	0.2
Head	0.20	0.56	0.76	29.5	0.5
Eye	0.19	0.54	0.76	30.2	1.7
Proboscis	0.23	0.43	0.67	31.2	12.0
Wing	0.16	0.54	0.72	34.7	4.6
Leg 1	0.15	0.52	0.71	33.1	10.6
Leg 2	0.16	0.56	0.70	35.0	5.0
Leg 3	0.22	0.54	0.70	35.3	2.8
Tergites 2 and 3	0.27	0.52	0.63	38.6	5.4
Non-gynandromorphic sibs (controls)	0.09		0.86		

The proportion of 532 gynandromorphic flies that consumed 1 M NaCl+0.1 M sucrose solution under standard experimental conditions is given, and the distance in sturt units between the focus of Lot-94 and the reference foci (Af) and between left and right foci of Lot-94 (ff') was calculated according to Hotta and Benzer¹², assuming that the mutant focus is submissive to the normal focus.

*Fate map distances change slightly and proportionally if corrected for consumption of non-gynandromorphic siblings.

sided tape on a microscope slide. Solution of sugar or mixed salt and sugar was applied from a capillary to alternating flies in the row, by touching their labellum or tarsi. The proportion of flies that extended their proboscis was registered. In other experiments, the labellum was touched and the length of time that the flies consumed the solutions was recorded (Table 1). There were no differences in the response to sugar between QA and Lot-94 when either the labellum or the tarsi were stimulated. Lot-94 flies were significantly less sensitive to salt when consumption time was measured. Reduced salt sensitivity was also indicated by extension of the proboscis on touching the labellum with salt solution. On the other hand, touching the tarsi with salt solution elicited less proboscis extension in the Lot-94 than in the wild-type QA flies. This indicates that tarsal reaction is not the decisive component of the flies' response in our standard testing procedure. Getting and Steinhardt⁹ postulated the existence of different intermediate neurones for the labellar and tarsal taste receptors in *Phormia*. The difference in labellar and tarsal response to salt in the mutant Lot-94 of *Drosophila* is consistent with this finding.

The behavioural pattern of a genetic mosaic depends on whether the genotype of the focus essential for the relevant phenotype is mutant or normal. In order to produce mosaics for Lot-94, females heterozygous for the unstable ring X-chromosome R(1)2, w^c were mated to $y w sn$ Lot-94/ y^+Y males¹⁰. In 25% of these heterozygous embryos, the unstable X-chromosome was lost in an early cleavage division, turning them into XX/XO gynandromorphs. Yellow cuticle (y), white eyes (w), singed bristles (sn), sex combs and male genitalia served to detect sectors of XO cells. These sectors were also hemizygous for the Lot-94 mutation. Since less than half of the gynandromorphs were mutant with respect to their taste response, the mutant focus was assumed to be submissive to

the normal. The focus of the mutant was located in the gynandromorphs on the basis of the assumption that the closer a cell was to the focus in the blastoderm, the higher the probability of agreement between their genotypes^{11,12}. The focus of Lot-94 was closer to that of the antenna than to that of the proboscis (Table 2). The only significant maximum likelihood estimate¹³ obtained so far, places the focus 31 sturts (see ref. 12 for definition) away from the proboscis and 6 sturts from the midline. This does not exclude the possibility that the focus of Lot-94 for taste perception of the labellum is located at a different site from that of the tarsi. The focus of Lot-94 was tentatively located midventrally, in the area of the blastoderm giving rise to the brain. Thus Lot-94 is a mutation of a perception centre, affecting the recognition of salt, with no detectable effect on that of sugar.

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Iridovirus and cytoplasmic polyhedrosis virus in the freshwater daphnid *Simocephalus expinosus*

AN iridovirus and a cytoplasmic polyhedrosis virus have been found as the cause of disease in the freshwater daphnid, *Simocephalus expinosus*, collected in Florida (Fig. 1). Although both types of virus were originally reported from insects¹⁻³ and are now well known insect virus types⁴, neither has been reported before from the Crustacea. Iridoviruses have been reported from other invertebrates and from some vertebrates⁵, however, cytoplasmic polyhedrosis viruses have been reported only from insects.

Daphnids infected with iridovirus were first collected during April 1972 from two woodland ponds, approximately 7 miles apart, near Gainesville, Florida. One pond was sampled periodically during each season of 1972, 1973 and 1974. *S. expinosus* seemed to be present only during the spring. During each sampling period, infected specimens were collected until the middle of June, after which host populations declined. The incidence of infected daphnids was never greater than 1%. All specimens were examined against a black background with a fluorescent light. Frankly infected daphnids were opalescent while apparently healthy specimens were grey and translucent.

For electron microscope studies, daphnids were dissected and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer for 3 h, postfixed in 1% osmium tetroxide for 2 h, dehydrated, and

embedded in an Epon-Araldite mixture, using standard methods. Examination of ultrathin sections of daphnids infected with iridovirus revealed virions throughout the adipose and glandular tissues, in the epidermis and associated endocuticle, and occasionally in nerve tissue. No virions were observed in muscle or gut epithelial tissues. Virus morphogenesis was restricted to the cytoplasm of infected cells. There was marked degradation of cellular organelles in advanced stages of the disease. Although large numbers of virions accumulated in the cytoplasm of many infected cells, paracrystalline arrays of virions, common in many iridovirus diseases, were not observed.

Structurally, virions were icosahedral and morphologically very similar to other virions of the iridovirus group⁵. Cross sections through mature virions indicated a dense central core (diameter about 96 nm), surrounded by at least one unit-membrane envelope which in turn was surrounded by the capsid. In most virions an amorphous layer of approximately 12 nm was observed between the envelope and the dense central core. The edge-to-edge and point-to-point diameters of mature virions were about 136 nm and 154 nm, respectively. From these measurements, an average fivefold rotational axis diameter of about 140 nm was calculated, using the icosahedral virus model of Mattern⁶. The virus tentatively has been designated iridovirus type 20, conforming with the interim nomenclature system for iridoviruses proposed by Tinsley and Kelley⁶.

The cytoplasmic polyhedrosis virus (CPV) was discovered during ultrastructural examinations of daphnids infected with iridovirus. This virus was only observed in the cytoplasm of the midgut epithelial cells. The occlusion bodies were pleiomorphic,

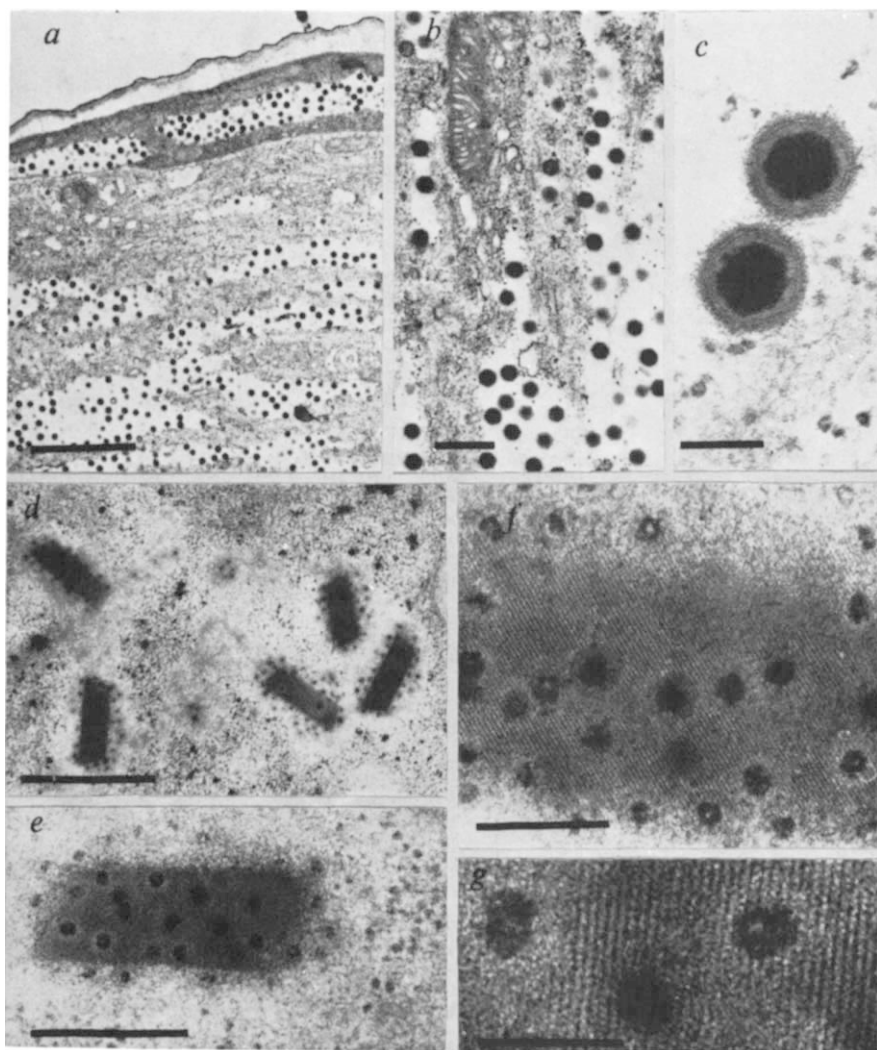


Fig. 1 Electron micrographs demonstrating iridovirus (a-c) in a portion of the post-abdomen and cytoplasmic polyhedrosis virus (d-g) in midgut (caecum) epithelial cells of the daphnid *Simocephalus expinosus*. a, Virions in epidermal and adipose tissue (bar=3 μ m); b, virions in the cytoplasm of an adipose cell (bar=500 nm); c, section through two mature virions illustrating the dense core and unit membrane interior to the capsid (bar=100 nm); d, oblong polyhedral occlusion bodies in the cytoplasm of a cell (bar=1 μ m); e and f, developing occlusion bodies. Note the virions with electron translucent centres toward the periphery of the occlusion bodies (e, bar=400 nm; f, bar=200 nm). g, Virion cores in an occlusion body (bar=100 nm). Note the crystalline lattice of the occlusion body in (f) and (g).

the smaller ones ($900 \times 300 \times 300$ nm) being brick-shaped polyhedra while the larger forms (up to 2 μ m) were spherical. Virion morphogenesis was different in that unlike other CPVs the virions apparently matured only in close proximity to developing occlusion bodies. Unoccluded virions were frequently observed with electron translucent centres. Occluded virions near the periphery of developing occlusion bodies also occasionally contained electron translucent centres, whereas those occluded within the central areas of large occlusion bodies had uniformly dense cores typical of unoccluded and occluded virions in other CPVs. The virions were icosahedral and approximately 60 nm in diameter. Because the capsid and occlusion body protein were similar in electron density and could not be easily distinguished, this figure should be considered tentative. The virion cores, which were quite distinct, were about 38 nm in diameter.

Couch⁸ reported a baculovirus, another type of virus found commonly in insects, in the pink shrimp, *Penaeus duorarum*. The observations reported here, in conjunction with Couch's report, demonstrate that virus types generally considered to be insect viruses, namely, baculoviruses, cytoplasmic polyhedrosis viruses, and iridoviruses, also have as their natural hosts members of the Crustacea. This is not to imply that insect viruses infect Crustacea, but rather that these virus groups have a broader taxonomic distribution than is generally realised. These findings indicate the need for further research directed toward determining the kinds of viruses which occur in Crustacea and other invertebrates. This is important especially because several pathogenic insect viruses are being considered as biological control agents. Knowledge of the distribution of virus types and their specificity in invertebrates will provide valuable background information for the implementation of biological control programmes involving insect viruses.

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Enzyme synergistic for insect viruses

A GRANULOSIS VIRUS (GV) and a nuclear-polyhedrosis virus (NPV) of the armyworm, *Pseudaletia unipuncta*, form a synergistic association in which the infection of the NPV is enhanced¹. The synergistic factor responsible for the enhancement (56-fold) occurs in the protein of the inclusion body (capsule) that surrounds the particle of GV^{2,3}. This factor is present in detectable quantities in one strain of GV (Hawaiian) but not in another (Oregonian)^{4,5}. It has been purified by Sephadex gel filtration and seems to be a simple or conjugated protein³. We now report that the synergistic factor has properties of an enzyme and can enhance not only the NPV and GV of the armyworm but also other insect viruses. Earlier studies^{1,2} had shown that the synergistic factor was heat labile. It became inactive when heated at 85 °C for 10 min (ref. 1). The viral activity, on the other hand, was destroyed at 75 °C for 10 min.

The synergistic factor seemed to be an enzyme because

it catalysed the hydrolysis of *p*-nitrophenyl esters of fatty acids and was inhibited by metallic ions. Tests for hydrolysis of the soluble substrates (esters of two to four carbon atoms) were made in a mixture containing the synergistic factor at 1 mg ml⁻¹, 5% isopropyl alcohol, 0.5 mM of the *p*-nitrophenyl ester, and 0.0025 M HCl-Tris buffer, pH 9.0 the pH of optimal hydrolysis. The mixture was held at 25 °C for 30 min, and the amount of product released was measured in the spectrophotometer at 410 nm, the wavelength of the peak absorption for *p*-nitrophenol. In the case of the insoluble substrates (esters of five or more carbon atoms), gum arabic at a final concentration of 1% was added as an emulsifier to stabilise the suspension. The mixture was agitated for 3 min in a sonicator. Subsequently, the treatment was essentially the same as for the soluble substrates, except that after incubation at 25 °C, chloroform was added, and the mixture was shaken for 3 min and centrifuged at 3,000 r.p.m. for 15 min to remove the residual insoluble substrate. The supernatant was collected and read at 410 nm. The controls, which consisted of the esters of the fatty acids, were examined concurrently with the treatments containing the synergistic factor.

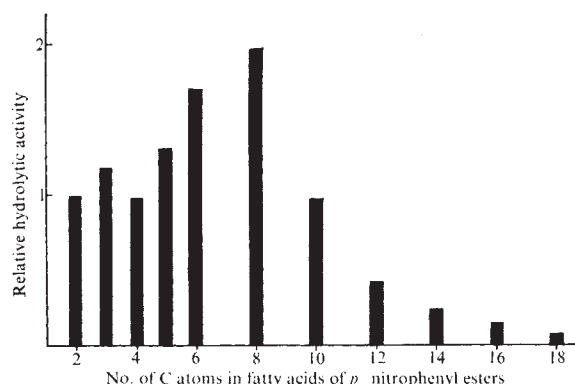


Fig. 1 Hydrolysis of fatty acids by the synergistic factor of a granulosis virus. The hydrolysis of *p*-nitrophenyl acetate is given a value of one. The synergistic factor was isolated and purified as follows. The GV capsules were dissolved largely by treatment for 3 h with 0.02 M NaOH at 4 °C, after which the insoluble material was removed by centrifugation at 20,000 r.p.m. for 1 h. The supernatant was dialysed against 0.01 M phosphate buffer, pH 8.0 and then concentrated in a collodion bag under reduced pressure at 4 °C. The concentrate was fractionated by filtration through a Sephadex G-200 gel at 4 °C, and the various components separated were examined with a Beckman DB-GT spectrophotometer at 280 nm. There were six peaks, and each was tested on fifth-instar armyworm larvae for its enhancing capacity as before². The synergistic factor occurred in the second peak fraction, which was concentrated at 4 °C in a collodion bag under reduced pressure and dialysed at 4 °C against 0.001 M phosphate buffer, pH 8, for 16 h. The dialysate was applied to a 1.5 × 5 cm hydroxylapatite column which had been equilibrated with 0.001 M phosphate buffer. The column was washed with 0.001 M phosphate buffer and the synergistic factor was eluted with 0.005 M phosphate buffer, pH 8. The eluate gave a single protein band in disc acrylamide gel electrophoresis using a 7.5% gel in a medium of 8 M urea and glycine buffer, pH 8.7.

In the controls, hydrolysis of the esters of fatty acids occurred also, but at a slower rate than in the presence of the synergistic factor. The results given in Fig. 1 are the differences between the controls and those obtained with the synergistic factor. The relative activity (with *p*-nitrophenyl acetate arbitrarily given a value of 1) of the synergistic factor increased as the carbon atoms in the chains of the fatty acids increased from two to eight, but possibly with a slight dip in activity with butyrate (four carbons). There was a gradual reduction in the hydrolysis of esters with 10 to 18 carbon atoms. Accordingly the synergistic factor catalyses the hydrolysis of synthetic esters of fatty acids.

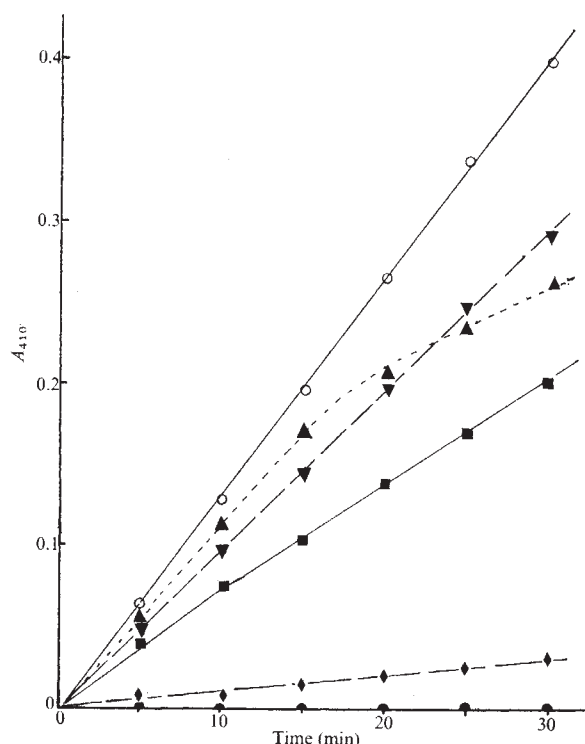


Fig. 2 Effect of metallic ions on the hydrolysis of *p*-nitrophenyl butyrate by the synergistic factor of a granulosis virus. ○, Control; ▼, Mn; ▲, Mg; ■, Ca; ◆, Cu; ●, Hg.

The optimum pH for the hydrolysis of *p*-nitrophenyl caprylate by the synergistic factor was studied at various pH using the following buffers: 0.05 M phosphate buffer for pH 6.0, 7.0 and 7.5; 0.05 M Tris-HCl buffer for pH 8.0, 8.5, and 9.0; 0.05 M carbonate buffer for pH 9.5, 10.0, and 11.0. The quantity of released *p*-nitrophenol was measured in the spectrophotometer at 410 nm. The peak in hydrolytic activity occurred at pH 9.

Since enzymes are often inhibited by metallic ions, the synergistic factor was exposed to five different salts of metals and its activity on *p*-nitrophenyl butyrate was examined. The salts were $MnCl_2$, $HgCl_2$, $CuSO_4$, $CaCl_2$ and $MgCl_2$, at 1 mM. These metals inhibited to varying degrees the hydrolysis of the butyrate by the synergistic factor (Fig. 2). Copper markedly inhibited and mercury completely inhibited hydrolysis.

The synergistic factor after exposure to these five metallic ions was tested for its enhancing capacity on NPV in the armyworm. After treatment with mercury, the enhancing capacity was destroyed; however, treatment with other metals had little or no effect on enhancement.

Before the isolation of the synergistic factor, our tests indicated that the GV was synergistic for the NPV of the armyworm but not for other insect viruses. But, when the synergistic factor was purified and concentrated and fed together with the GV (Hawaiian and Oregonian strains) of the armyworm, with the NPV and GV of the cabbage looper (*Trichoplusia ni*), and of the beet armyworm (*Spodoptera exigua*), it enhanced the infection of these viruses.

The inclusion bodies, such as the polyhedra of the NPV and of the cytoplasmic polyhedrosis virus (CPV), the capsules of GV, and spherules of poxvirus, are generally believed to protect the virus particles occluded within them from the adverse effects of the environment. The presence of the synergistic factor within the capsule of the GV suggests another function, that of enhancing the infection of the virus.

Although the shapes of inclusion bodies, such as the polyhedra of NPV⁶⁻⁹ and of CPV^{8,10-13} and the capsule of GV¹⁴, are apparently determined by the virus, the origin

of these proteins, whether virus- or host-directed, has not been established. The origin of the enhancing enzyme (synergistic factor) is still to be determined, but its detectable presence only in the Hawaiian GV strain suggests that it is a virus-directed protein*.

Recently, the use of chemical insecticides has been encouraged less than before, primarily because of pollution of the environment, the development of resistance in insect pests, the adverse effect on insect parasites and predators, and the conversion of minor pests to major economic pests. One of the areas receiving increasing interest is microbial control where pathogens, such as viruses, bacteria, fungi and protozoa, are used for the control of insect pests. In as much as the synergistic factor enhances not only the NPV of the armyworm but also other insect viruses, it is potentially important in microbial control of insect pests.

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*Note added in proof: Dr M. D. Summers, University of Texas at Austin, has informed us of his discovery of an alkaline protease enzyme in the polyhedra of an NPV of *Trichoplusia ni*.

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Experimental viral labyrinthitis

CONGENITAL and acquired deafness and acute and chronic vertigo are often attributed to viral infections of the inner ear. Direct demonstration of natural or experimental infection of the membranous labyrinths of the cochlear and vestibular systems has not been practicable, however, because these structures are encased in the dense temporal bones. Evidence that viral infections can cause deafness and vertigo in man is based largely on epidemiological observations and on a limited number of temporal bones examined months or years after the acute event. Temporal bones from patients with deafness associated with mumps, measles, cytomegalovirus and rubella have shown a cochlear-saccular degeneration suggesting that endolymphatic structures may have been infected¹⁻⁴. Except for cytomegalovirus infections, which cause characteristic histopathological changes⁴, there are no previous studies of acute viral infections of the inner ear in patients or experimental animals.

We have now studied the pathogenesis of acute viral infections of the inner ear in newborn hamsters, whose temporal bones are not calcified at birth. This made possible the use of fluorescence microscopy to localise infection at a cellular level after direct intralabyrinthine or intracerebral viral inoculations. The distribution of inocula was first localised by the injection of India ink. Following intracerebral inoculation, carbon

particles reached the cochlear scala tympani from the subarachnoid space through the cochlear aqueduct, which is patent in rodents⁶. Carbon particles were seen in perilymph but not endolymph. A few particles also reached the perineural and perivascular spaces of the cochlear modiolus through the internal auditory canal. Direct intralabyrinthine inoculations were performed percutaneously using a microsyringe with a 30-gauge needle. Volumes of 0.005 ml could consistently be introduced through the temporal cartilage into the labyrinth. Injections of India ink showed that carbon particles were variably distributed into the endolymph, perilymph or both.

Three viruses were studied. The mouse-adapted neurotropic (NWS) strain of influenza A virus⁷, prepared as a 10% homogenate of infected mouse brains, had an infectivity of $10^{5.6}$ —50% lethal doses (LD_{50}) per ml determined by intracerebral inoculation of newborn hamsters. The hamster-adapted Kilham strain of mumps virus⁸, grown in Vero cells, had an infectivity of $10^{6.2}$ —50% tissue culture doses (TCD_{50}) per ml. The hamster-adapted neurotropic strain of measles virus (HNT)⁹, prepared as a 10% homogenate of infected suckling hamster brains, had an infectivity of $10^{6.3}$ — LD_{50} per ml determined by intracerebral inoculation of newborn hamsters. Virus pools were diluted 1:10 in balanced salt solution, and 0.005 ml of these dilutions was inoculated into each labyrinth or 0.02 ml

was inoculated intracerebrally.

30 min. Rhodamine was used as a counterstain. After final washings, the sections were mounted in neutral glycerol and examined by fluorescence microscopy.

Influenza A virus inoculated by either intracerebral or intralabyrinthine routes resulted in a similar pattern of infection. Immunofluorescent staining demonstrated viral antigen in the loose mesenchymal cells of the cochlear aqueduct and scala tympani 1–4 d after inoculation (Fig. 1a). Eighth nerve ganglion cells in the cochlear (spiral) ganglia and vestibular (Scarpas) ganglia also showed specific fluorescence. No viral antigen was found in endolymphatic structures.

In contrast, mumps virus infected endolymphatic structures. This infection was most consistent after intralabyrinthine inoculation but occasionally some endolymphatic cells contained antigen after intracerebral inoculation. Nine to 12 d after inoculation, cells in both layers of Reisner's membrane, the stria vascularis, and the organ of Corti contained mumps virus antigen (Fig. 1b). Specific fluorescence was also seen in cells of the macula of the saccule and adjacent endolymphatic membrane. Again neurones in both the cochlear and vestibular ganglia stained specifically, but no viral antigen was seen in perilymphatic structures.

Measles virus inoculated by either route infected both

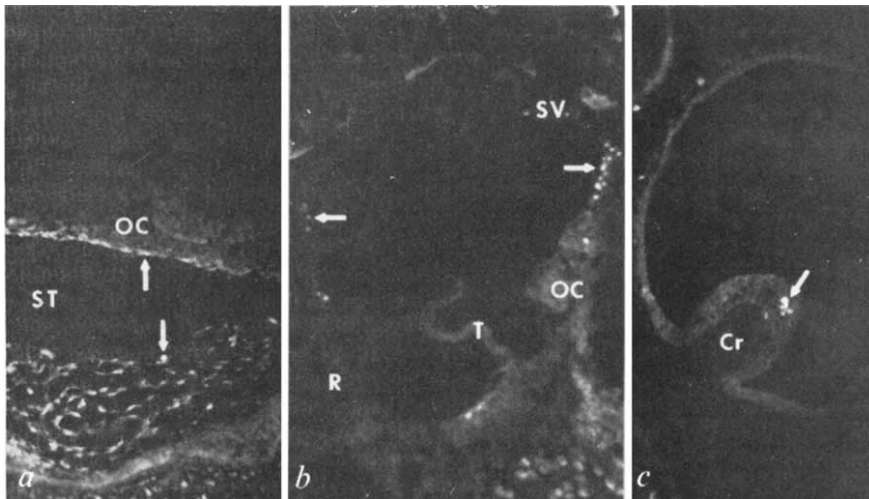


Fig. 1 Micrographs of hamster inner ears stained with fluorescent antibodies. *a*, Cochlea 1 d after intracerebral inoculation of influenza A virus. The arrows indicate the location of specific fluorescence in the loose mesenchymal cells of the perilymphatic scala tympani (ST) below the immature organ of Corti (OC). ($\times 78$). *b*, Cochlea 11 d after intralabyrinthine inoculation of mumps virus. The arrows indicate the location of viral antigen in the organ of Corti (OC), stria vascularis (SV), and Reisner's membrane (R), but not in the tectorial membrane (T) ($\times 78$). *c*, Semi-circular canal 3 d after intralabyrinthine inoculation of measles virus. The arrow shows viral antigen in the crista (Cr) ($\times 78$).

Newborn hamsters were observed for signs of illness following intracerebral or intralabyrinthine viral inoculation. Since hamsters do not have functioning inner ears before day 20 (ref. 10), no clinical signs of inner ear disturbance could be observed. Animals inoculated by either route also developed encephalitis 3–4 d after inoculation of influenza or measles virus and 9–13 d after inoculation of mumps virus. Litters inoculated with diluent remained well and were killed at appropriate times for controls.

After the animals had been killed, optimal preservation of inner ear structure and viral antigens was achieved by using saline perfusion followed by perfusion of cold 95% ethanol. The skinned head, with jaw removed, was fixed in 95% ethanol for 24 h at 4 °C, dehydrated in graded steps of cold 100% ethanol, cleared in cold xylene and embedded in paraffin wax according to the method of Sainte-Marie¹¹. Serial horizontal sections of the head including both inner ears were cut on a rotary microtome. At various levels of the inner ears adjacent sections were taken for histological examination and immunofluorescence studies. The paraffin sections were stored at 4 °C until processed for fluorescent antibody staining. The paraffin was removed by washing in consecutive cold xylene, alcohol and saline baths. Sections were then overlaid with virus-specific guinea pig antisera or with control non-immune sera for 30 min. Sections were then washed in saline and overlaid with fluorescein-conjugated rabbit anti-guinea pig γ globulin for

perilymphatic cells and cells in the organ of Corti and crista. Three and four days after inoculation, loose mesenchymal cells in the cochlear aqueduct and scala tympani of the cochlea contained viral antigen. Measles antigen in membranous labyrinth was limited to cells in the organ of Corti and crista of the semi-circular canals (Fig. 1c). The cells of the surrounding endolymphatic membrane were not involved. Again neurones of the cochlear and vestibular ganglia stained specifically.

Sections of saline-inoculated control animals showed no specific fluorescence when stained with viral antisera, and sections of all virus-inoculated animals were negative when stained with non-immune antisera.

Histological examination of sections stained with haematoxylin and eosin showed only minimal inflammatory responses elicited by influenza and mumps virus. Measles virus, however, caused giant cell formation in the spiral ganglia and occasionally in the organ of Corti.

These studies have demonstrated the feasibility of infecting inner ear structures in an experimental animal either by direct inoculation into the labyrinth or indirectly from the subarachnoid space through the cochlear aqueduct. Endolymphatic structures are, however, more readily infected using percutaneous intralabyrinthine inoculation. Preservation of the delicate membranous structures of the inner ear as well as preservation of viral antigens has been possible using paraffin embedding following cold alcohol fixation.

These initial studies have shown that cells of the inner ear differ in their vulnerability to viral infections. Each virus caused a different pattern of infection: influenza virus infection was limited to perilymphatic structures, mumps virus to endolymphatic cells, and measles virus involved both perilymphatic cells and the organ of Corti and the crista. All three viruses infected ganglion cells of the eighth cranial nerve. It is significant that both measles and mumps viruses infected endolymphatic structures or cells in the organ of Corti, since an endolymphatic labyrinthitis has been implicated as the original cause of damage in patients with deafness associated with these two virus infections¹⁻³.

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Effect of electric fields on growth rate of embryonic chick tibiae *in vitro*

NUMEROUS investigators have reported work on the stimulation of bone growth by electric currents in the microampere range, with varying degrees of success. Such work, along with the various tentative theories of the underlying mechanisms, have been summarised by Bassett¹, and some quantification of the effect has been attempted^{2,3}. Hambury *et al.*³ concluded that the necessary crudity of the surgical techniques involved, and the overriding effects of the trauma militated too severely against accurate quantification of the electrically-stimulated bone growth, and proposed⁴ that *in vitro* experiments might yield more definitive results. They also concurred with other workers that if electric currents do influence bone growth, then so should electric and magnetic fields, and Norton⁵ has already performed work in this area *in vivo*, using chickens. We report that the gross development of embryonic chick tibiae grown *in vitro* is affected by a pulsed, transverse electric field, but that no significant changes were observed when a static, non-varying field was used.

Between one and three bones were placed in each of eight Petri dishes, four of the dishes being subjected to a 1,000 V cm⁻¹ electric field, and four being placed between the inactive plates of a second control apparatus (see Table 1 for detailed method). Each bone was measured before and after a 9 d incubation period. The ratio of the final to the initial bone length was calculated for each bone, giving the fractional increase in length in each case. Also, the experiments were systematised so that the two ratios obtained for the bones from each embryo could be related and compared. Where bones distorted somewhat, the lengths of the longitudinal median lines were measured incrementally. Table 1 gives the results in terms of the fractional increase in length

Table 1 Effect of an invariant electric field on embryonic chick tibiae growth *in vitro*

Batch	Fractional length increase			'One-tail' t test		
	Test	Control	Control	t	d.f.	Confidence limit
A	1.50	1.50	1.00	1.52	6	> 5% (less growth)
	1.42	1.57	0.90			
	1.64	1.92	0.85			
	1.29	1.43	0.90			
	1.67	1.67	1.00			
	1.58	1.67	0.86			
B	1.46	1.26	1.16	1.35	7	> 10%
	1.27	1.24	1.02			
	1.31	1.21	1.08			
	1.18	1.25	0.94			
	1.34	1.28	1.05			
	1.34	1.25	1.07			
	1.58	1.44	1.04			
C	1.43	1.38	1.04	0.68	7	Not significant
	1.46	1.60	0.91			
	1.43	1.48	0.97			
	1.42	1.37	1.04			
	1.43	1.50	0.95			
	1.57	1.48	1.06			
	1.49	1.44	1.03			
D	1.44	1.41	1.02	1.67	7	> 5%
	1.30	1.47	0.88			
	1.59	1.34	1.48			
	1.41	1.48	0.95			
	1.48	1.44	1.03			
	1.43	1.45	0.99			
	1.64	1.21	1.36			
E	1.50	1.51	0.99	0.65	6	Not significant
	1.39	1.35	1.03			
	1.46	1.46	1.00			
	1.30	1.16	1.12			
	1.19	1.16	1.03			
	1.19	1.15	1.03			
	1.20	1.19	1.01			
Overall	1.14	1.09	1.05	0.21	37	Not significant
	1.20	1.21	0.99			
	1.14	1.26	0.90			

The 8-9-d-old embryos were aseptically decapitated, and extracted and the tibiae carefully excised. Each bone was then placed on a thin siliconised cellulose acetate mat supported by a 3 mm thick sponge of similar material, both items being chemically clean and sterile. Each assembly, consisting of a sponge, mat and two or three bones was placed in a 35 mm disposable polystyrene Petri dish containing sufficient medium to thoroughly soak the sponge without immersing the bone. The bones must be allowed free exposure to the incubator atmosphere of CO₂-air (5:95). Chemically-defined BGJ-b nutrient medium⁶ was used, as described previously. In particular, the medium was changed every 2 d by gently removing the cellulose mat and placing it on a new sponge in a new Petri dish. This enabled most of the metabolic by-products of the preceding 2 d to be removed in the interstices of the sponge, the mat to which the bones adhered being too thin to retain more than a very small portion of these products. The bones were incubated for 9 d at 37 °C, after which they reached their maximum length. This period was defined by trials in which the length of each bone was measured and plotted against time until the graph became horizontally asymptotic. These measurements were made outside the Petri dishes using a micrometer scale mounted on a dissecting microscope eyepiece. After excision, one bone from each embryo was placed in a Petri dish inserted between the plates of the apparatus applying the electrical field. The other bone was placed in a dish within a similar, but inactive, apparatus as control. Both sets were placed in the same incubator. The apparatus for applying the field consisted essentially of two circular stainless steel plates, 12.7 cm in diameter, and held 1.4 cm apart by a Perspex framework. A Keithley model 244 high voltage power supply was used to apply 1,400 V across the plates, so that a uniform invariant field of 1,000 V cm⁻¹ existed between them, the lower plate being grounded. A Plexiglass disk, 3 mm thick, drilled with four holes 38 mm in diameter, was used to slide the four 35 mm Petri dishes into the space between the stainless steel plates. In the fully operating system, an interlock microswitch was incorporated into the incubator so that the opening of the door switched off the power supply. The stainless steel plates of the second apparatus, containing the controls, were short-circuited and grounded.

of each bone subjected to the field, compared with that for each corresponding control bone from the same embryo. The ratio of each pair of fractional increases is also included. Further, a 'one-tail' *t* test was applied to each batch, and also to the complete series. This shows that there is no significant difference between the test and control bone fractional increases.

A second series of experiments was carried out using a $1,000 \text{ V cm}^{-1}$ field pulsed at approximately 1 pulse s^{-1} using a high voltage relay driven at this pulse repetition rate by a unijunction transistor circuit. The live (upper) plate was alternately connected to the $-1,400 \text{ V}$ supply and to ground by this means, so that a good square wave of approximately 1:10 mark-space ratio was obtained. Table 2 shows that for batch A, the chances that the pulsed electric field had no effect were no more than 0.5%; and for batches C and E, were no more than 2.5%. These results can therefore be considered significant, whereas those for batches B and D could not, being more than 10 and 25%, respectively. The 'one-tail' *t* test was also applied to the entire series taken as a whole (Table 2). The overall confidence limit was less than 0.5%, clearly very significant.

Bones were photographed in their Petri dishes at the end of each test to provide a record of their macroscopic appearance, then fixed in neutral formalin for 3 d. After manual processing, they were embedded in paraffin wax and sectioned longitudinally. The sections were then fixed to

glass slides by means of a glycerin-albumin mix and dewaxed in a hot air oven. They were then regressively stained with haematoxylin, counterstained with eosin, and examined with an optical microscope. This did not reveal any differences between test and control slides; nor did a macroscopic inspection of the photographs reveal any differences in growth patterns.

We propose to grow further batches and perform more comprehensive tests including cell counts, wet and dry weight measurements and DNA content analyses, and to determine the effect of variation in field strength, frequency, waveform, and the spatial relationship of the field to the bone.

If our trials are significant, it can be concluded that there is present in the embryonic bone a transducer mechanism which allows the electric field to interact directly and modify (at least) the growth rate. Since the bones are in a pre-osseous phase, this mechanism clearly cannot be related to any properties of hydroxyapatite, thus ruling out at least one mechanism, the collagen-hydroxyapatite semiconductor interface¹. In addition, because only pulsed electric fields (as opposed to invariant fields) have an effect, it is likely that repeated cycles of charge separation are involved. This is equivalent to applying very small pulsed currents, and since such currents can also be induced by pulsed magnetic fields, a series of experiments using such fields has been initiated. Note that Bassett *et al.* have reported that pulsed magnetic fields do modify bone growth patterns *in vivo*⁷.

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Table 2 Effect of a pulsed electric field on embryonic chick tibiae growth *in vitro*

Batch	Fractional length increase			'One-tail' <i>t</i> test		
	Test	Control	Test/control	<i>t</i>	d.f.	Confidence limit
A	2.56	1.64	1.56	4.49	6	<0.5%
	2.70	2.10	1.29			
	2.36	1.86	1.27			
	2.62	2.21	1.24			
	2.32	2.25	1.03			
	2.40	2.17	1.11			
	2.50	1.87	1.34			
B	2.66	2.25	1.18	1.24	7	>10%
	2.40	2.14	1.12			
	2.24	2.15	1.04			
	2.15	2.00	1.07			
	2.00	1.91	1.05			
	2.30	2.43	0.95			
	2.43	2.09	1.16			
C	2.05	2.24	0.92	3.32	7	<2.5%
	1.80	1.45	1.24			
	2.11	1.50	1.41			
	1.68	1.65	1.02			
	1.62	1.43	1.13			
	2.06	1.37	1.50			
	1.83	1.62	1.13			
D	1.74	1.65	1.05	0.58	9	>25%
	1.83	1.78	1.03			
	1.67	1.52	1.10			
	1.45	1.50	0.97			
	1.74	1.67	1.04			
	1.67	1.74	0.96			
	1.32	1.57	0.84			
E	1.39	1.61	0.86	2.80	7	<2.5%
	1.56	1.05	1.49			
	1.58	1.72	0.92			
	1.67	1.25	1.34			
	1.64	1.60	1.02			
	2.00	1.96	1.02			
	2.04	1.92	1.06			
Overall	2.03	1.74	1.17	2.81	40	<0.5%
	2.36	1.68	1.40			
	2.00	2.03	0.99			
	1.90	1.87	1.02			
	2.22	1.82	1.22			
	1.90	1.76	1.08			
	2.01	1.80	1.12			

Left-handed to right-handed helix conversion in *Salmonella* flagella

ALTHOUGH bacterial flagella can assume various helical configurations¹⁻³, the 'normal' and 'curly' configurations are encountered most frequently in nature. In *Salmonella*, normal and curly flagella have helical pitches of about 2.3 and 1.1 μm , respectively⁴. Macnab and Koshland⁵ observed that normal flagella of living *Salmonella* were left-handed helical filaments, as shown for *Proteus* and *Bacillus*⁶. Flagella with this handedness and rotating counterclockwise (looking in the direction of travel), would cause helical waves to propagate distally and provide forward thrust⁷⁻⁹. Normal flagella can transform reversibly into the curly type when physiological conditions, such as pH, are varied^{10,11}; this has been termed biplicity. Asakura *et al.*¹², using *Salmonella* strain SJ670 and others, found that reconstituted flagellar filaments also could be transformed reversibly, although they could not control this transformation completely. SJ670 is a motile strain that produces

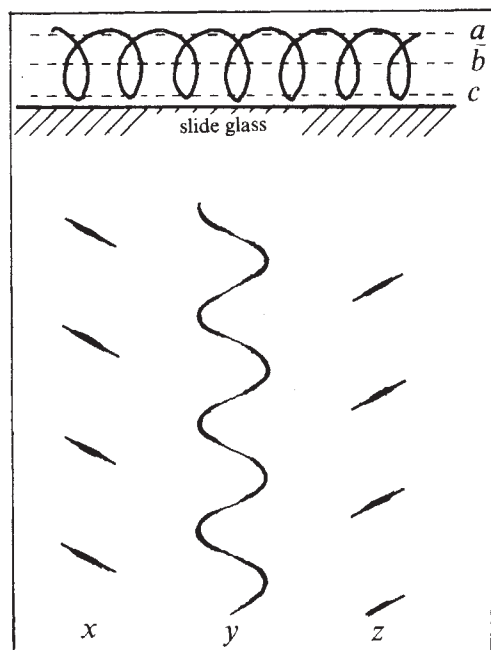


Fig. 1 The optical method used for the determination of helical handedness. For explanation, see text.

normal flagella. On the other hand, the mutant SJ30 isolated by Iino¹³ produces flagella which are stable in the curly configuration in various conditions. The mutation is in the structural gene for the flagellar protein subunit, flagellin, and the mutant cannot swim normally but appears to tumble continuously. Now, using SJ670 and SJ30, we have found that, whereas the normal configuration of flagella is a left-handed helix, the curly configuration is a right-handed helix.

Flagellins were purified from the two strains and polymerised into flagellar filaments as before¹². Flagellins of SJ670 and SJ30 repolymerised into normal and curly filaments, respectively, which were used in the experiments. We also used curly filaments obtained by copolymerising flagellins of SJ670 and SJ30 at a protein ratio of 9:1¹⁴. Other curly filaments used were composed of SJ670 flagellin at pH 4.2, for we have found that such filaments are transformed reversibly when the pH changes from 7.0 to 4.2. (Full details will be reported elsewhere.)

If flagellar filaments are suspended in a medium containing methylcellulose (about 0.5% w/v), they associate into bundles visible by dark-field light microscopy, and heterogeneous in thickness (as judged by brightness) and overall length. They are helix-shaped, however, with a pitch equal to that reported by Pijper *et al.*⁴ (Table 1). We have determined the helical handedness of bundles, because of

the difficulty of photographing individual filaments, assuming that the helical form of individual filaments changed little with association. The optical system consisted of an Ushio 100 W mercury arc lamp, and a Nikon SKR microscope equipped with an Olympus d.c. condenser (numerical aperture 1.2–1.33), an Olympus Apo 40 × objective (numerical aperture 1.0), Nikon 20 × eyepiece and a Nikon EFM camera. In this system, there are no beam reflections and therefore micrographs are real, not mirror images.

The method used for the determination of helical handedness can be understood by considering a bundle of left-handed helical filaments, lying on the surface of a slide glass (Fig. 1). If parts of the bundle near planes *a*, *b* and *c* are illuminated successively and focused by raising the specimen stage discontinuously, then images of types *x*, *y* and *z* will be obtained in succession. If a bundle of right-handed helical filaments is examined, images of types *z*, *y* and *x* will be observed, corresponding to planes *a*, *b* and *c*, respectively.

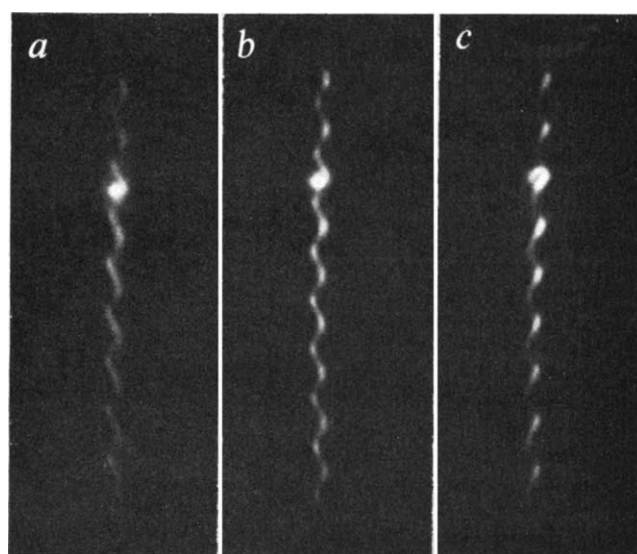


Fig. 2 A set of micrographs obtained for a bundle of normal-type filaments (SJ670) at pH 7.0. Micrographs (*a*), (*b*) and (*c*) were obtained by successively raising the specimen stage. The scale represents 5 μ m.

Figure 2 shows a set of micrographs obtained for a bundle of normal filaments. Clearly the bundle had a left-handed helical form. From this and many other observations we confirmed that normal flagella are left-handed helical filaments. In contrast, curly filaments of each kind examined were found to have a right-handed helical form (Fig. 3). Previously normal and curly configurations of flagella were believed to have the same helical handedness^{3,14}. It is important that helical handedness is reversed when filaments transform from normal to curly when the pH changes, for this means that the same protein can form filaments with left or right-handed helical symmetry.

Others have argued that in a genus such as *Salmonella* and *Escherichia*, in which the cells are peritrichously flagellated, each organism swims by rotation of the left-handed helical flagella in a counterclockwise sense and tumbles by reversal of the sense of flagellar rotation^{7–9}. This argument is based on the observations of the manner in which tethered bacteria swim. We observed that bacteria of SJ30 tethered on to the surface of a slide glass also spin counterclockwise. Therefore, it is likely that in SJ30, the right-handed helical flagella are rotating counterclockwise, giving rise to reverse thrust and continuous tumbling. At a recent meeting (First International Congress of the International Association of Microbiological Societies, Tokyo, September

Table 1 Four specimens examined

Flagella	pH	Concentration of added NaCl	Helical pitch* (μ m)	Type	Helical handedness
SJ670	7.0	0.01 M	2.35	Normal	Left
SJ670	4.2	0.3 M	1.13	Curly	Right
SJ30	7.0	0.01 M	0.99	Curly	Right
9:1 Copolymer of SJ670 and SJ30	7.0	0.01 M	1.04	Curly	Right

Each sample solution was prepared as follows. To a flagella solution containing 0.1 mg ml⁻¹ protein, 0.01 or 0.3 M NaCl, 5 mM citrate and 10 mM Na₂HPO₄ was added 2 N NaOH or HCl to give pH 7.0 or 4.2, and then the solution was mixed with an equal volume of a solution containing 1% methylcellulose and 0.01 or 0.3 M NaCl.

* Experimental errors were less than 5%.

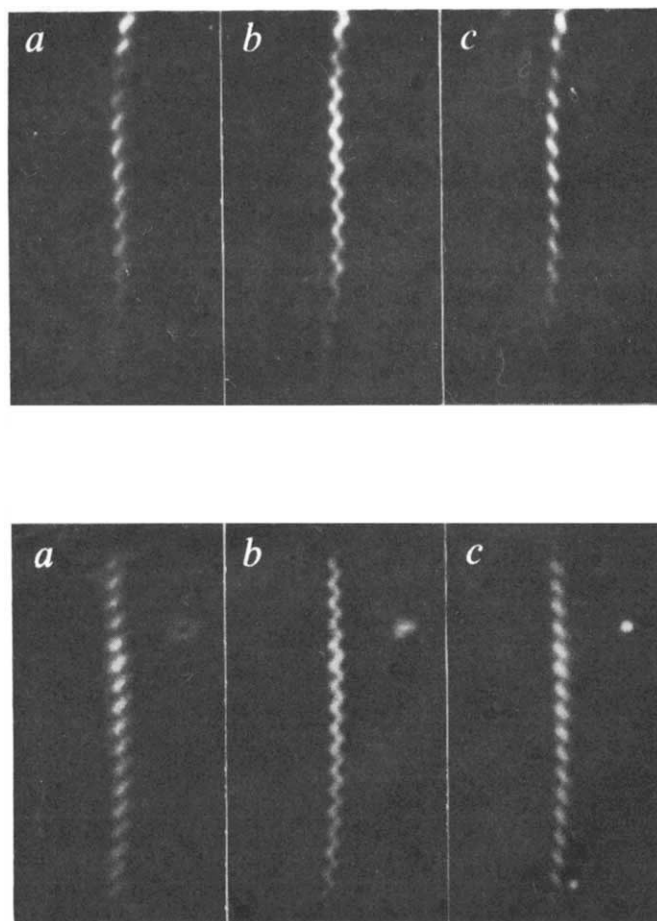


Fig. 3 Two sets of micrographs obtained for bundles of curly-type filaments: SJ670 (wild type) at pH 4.2 (top) and SJ30 curly mutant at pH 7.0 (bottom). In each set, micrographs (a), (b) and (c) were obtained by successively raising the specimen stage. The scale represents 5 μ m.

1974), R. M. Macnab reported that during bacterial tumbling induced by high intensity blue light⁵, or during spontaneous tumbling in uncoordinated mutants, normal filaments were transformed to the curly configuration. Also, in spite of the findings that reversal of rotation occurs during tumbling⁷⁻⁹, wave propagation was always observed to proceed distally. From these observations he predicted that transformation from normal to curly by hydrodynamic forces was accompanied by a left-handed to right-handed helix conversion, in agreement with our observations. Macnab's finding suggests that the conversion from normal to curly (left-handed to right-handed) configuration of flagella plays a role in the mechanism of tumbling and therefore tactic responses.

It is well known that when peritrichously flagellated bacteria swim in a medium containing methylcellulose, the flagella attached to each cell associate into bundles each with the appearance of a rotating helix. We have found that reconstituted flagellar filaments also associate into bundles in the presence of methylcellulose, though the nature of inter-filament interaction is not well understood. Asakura and Oosawa¹⁵ pointed out that if a macromolecular substance (in this case, methylcellulose) is added to a suspension of large particles (flagellar filaments), an attractive force appears between particles, even when there is no specific interaction between macromolecule and particle. This force is of entropic origin, since the volume which the added macromolecules can occupy is increased to some extent if the particles aggregate. This kind of force may be involved in the inter-filament interaction.

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Prevention of induced leukaemia in mice by immunological inhibition of adenohypophysis

THE theoretical and technical approach to an immunological inhibition of the adenohypophysis (AH) has been discussed in previous work^{1,2}. It has been shown that heterologous antibodies against mouse or rat AH have high organ-specificity and affinity for acidophilic cells of the anterior pituitary gland producing growth hormone. The possible application of such an immune intervention in the prophylaxis and therapy of hormone-dependent or hormone-sensitive tumours has been suggested¹ and experiments have demonstrated that hormone-dependent mammary carcinoma in rats can be prevented by anti-AH serum treatment³.

Further, the thymus is primarily involved in experimental leukaemogenesis in mice and thymectomy prevents or delays the onset of leukaemia induced by X rays or chemical carcinogen^{4,5}. In view of the extraordinary sensitivity of the thymus to hormonal change in general and especially to that of growth hormone⁶, we suggested that inhibition of cells of the AH secreting growth hormone (or prolactin) might effect lymphoid cell turnover in the thymus and therefore influence the onset of leukaemia¹. Recently, endocrine derangements have been found in SJL/J mice, a strain characterised by a very high incidence of spontaneous reticulum cell neoplasms (RCN), and this observation prompted the present investigation on the role of hormone in leukaemogenesis⁷. We have therefore investigated the effect of anti-AH serum in mice treated with different leukaemia-inducing agents.

Three groups of C57BL/6 inbred female mice (50 d old) received four doses of 170 rad wholebody irradiation at weekly intervals. At 34 d after the first irradiation, group 1 was injected intraperitoneally with 0.5 ml of sterile sheep anti-C57BL/6 AH serum, prepared and titrated as described previously^{1,8}. Group 2 was inoculated with the same amount of normal sheep serum (NSS) and group 3 was untreated. The same quantities of anti-AH serum or NSS were sub-

Table 1 Effect of anti-AH serum on lymphosarcoma induction by X rays in C57BL/6 mice

Host treatment	Lymphosarcoma incidence	Average latent period (d)
Anti-AH serum	6/19 (31%)	245
NSS	19/22 (86%)	201
—	11/14 (78%)	198

sequently inoculated 48, 66, 96, 175 and 210 d after the first irradiation.

Using previously described techniques⁹, three groups of SJL/J female mice (50 d old) were administered by stomach tube five doses of the carcinogen 7,12-dimethylbenzanthracene (DMBA) at weekly intervals. A dose of 0.1 ml of the DMBA solution (1 mg DMBA) in polyethylene glycol 400 was given at each feeding. At 31 d after the first feeding, one group was injected intraperitoneally with 0.5 ml anti-AH serum, one group was injected with the same quantity of NSS and one group was untreated. At 34, 48, 71, 93, 118, and 142 d after the first DMBA feeding, further doses of 0.5 ml anti-AH serum or NSS were injected. Because of the danger of anaphylactic shock in SJL/J mice, which are exceedingly susceptible to sensitisation with heterologous proteins, increasing doses (0.2; 0.4 ml of serum diluted 1:10; 0.1 ml undiluted serum) were

Table 2 Effect of anti-AH serum on lymphosarcoma induction by DMBA in SJL/J mice

Host treatment	Lymphosarcoma Incidence	Average latent period (d)	Reticulum cell neoplasms Incidence	Average latent period (d)	Other tumours
Anti-AH serum	12/39 (31%)	227	8/39 (20%)	299	2/39 (5%)
NSS	27/35 (77%)	134	2/35 (5%)	320	1/35 (2%)
—	9/15 (60%)	161	2/15 (13%)	253	3/15 (20%)

given respectively 3, 2 and 1 d before the final dose of 0.5 ml anti-AH serum or NSS.

Both treatments of either X rays in C57BL/6 mice¹⁰ or DMBA in SJL/J mice⁹ induce high incidences of lymphosarcomas. Both X rays and DMBA had no effect on the development of spontaneous RCN. Tables 1 and 2 show that inoculation with anti-AH antibodies during and after treatment with carcinogenic agents drastically reduced the incidence of lymphosarcoma in both experimental models. Further, the antiserum prolonged the average latent period for the development of the disease. On the other hand, treatment with anti-AH serum (Table 2) did not influence development of RCN in SJL/J mice.

Many direct and indirect data support the hypothesis that host factors of endocrine character are of primary relevance for development of systemic neoplasia^{7,11}. Our data support this contention by demonstrating that immunological inhibition of the adenohypophysis influences both the incidence and time of onset of lymphosarcoma in mice.

The mean latent period for spontaneous RCN is beyond 400 d and that for lymphatic leukaemia in DMBA-treated SJL/J mice is 130–170 d. The low incidence of RCN in the experiment with SJL/J mice (Table 2) probably results from the fact that the experiment was terminated after one year. Anti-AH serum does not influence incidence and does not delay onset of spontaneous RCN in SJL/J mice (W. P. and N. H-G., unpublished), thus pointing out the remarkable selectivity of action of anti-AH serum for lymphosarcoma. Both organ-specificity and potency of the anti-AH antibodies can undoubtedly be enhanced by further studies. It is therefore most astonishing that such a striking reduction in incidence of leukaemia was obtained in both experimental models in view of the relatively empirical dosage of the antibodies. It will be of particular interest to determine whether this distinctive kind of immune intervention will be similarly effective in preventing naturally occurring leukaemia, such as that in AKR mice. These data encourage new approaches to therapy of lymphatic leukaemia by 'immunological hypophysectomy'

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Oxygen dissociation properties of human embryonic red cells

THERE are three stages in the development of human haemoglobins. In the smallest embryos studied the embryonic haemoglobins, Hb-Gower 1, Hb-Gower 2 and Hb-Portland are the main haemoglobins found¹, but foetal haemoglobin (Hb-F) is also present in gradually increasing amounts. By about the 30 mm (crown-rump (CR) length) stage, Hb-F amounts to about 50% of total haemoglobin and after 50 mm it forms over 90% of total pigment. Adult haemoglobin (Hb-A) is already present by week 8 of gestation but this does not exceed 10% of total haemoglobin until week 30; after this it gradually increases until at term it forms between 10% and 30%. After birth, Hb-F is rapidly replaced by Hb-A and at 6 months Hb-F is less than 10%, while at one year it forms less than 2% of total haemoglobins. The oxygen dissociation properties of adult haemoglobin and of foetal haemoglobin have been studied in great detail. Foetal blood has a higher oxygen affinity than adult blood; this helps the transport of oxygen across the placenta and ensures that the steep part of the oxygen dissociation curve corresponds to foetal tissue oxygen tensions, thus maximising the release of oxygen. The oxygen affinities of the human embryonic haemoglobins have so far not been studied because the amounts available have been extremely small. Here we describe the oxygen dissociation properties of human embryonic red cells which were found to be similar to those of foetal red cells.

Cord blood samples were taken from embryos and foetuses obtained from terminations of pregnancy carried out for unrelated maternal indications. Great care was taken to avoid contamination by maternal blood and the electrophoretic patterns obtained confirmed that this was not a problem. The red cells were washed three times in isotonic NaCl and the oxygen dissociation curves of the red cells suspended in isotonic phosphate buffers pH 7.13 (or pH 7.46) at 37 °C were measured². In the method used the saturation of the haemoglobin with oxygen is measured spectrophotometrically using the second sample position of a Unicam SP 1800 or SP 800(0) recording spectrophotometer. Because of the small amount of cells the absorbance scale expansion was used. First the haemoglobin was deoxygenated using a vacuum and the spectrum recorded, then a measured amount of air was let into the tonometer and after equilibration at 37 °C the spectrum was again recorded. This procedure was repeated until the haemoglobin was fully saturated with oxygen. The partial pressure of oxygen P_{O_2} in the tonometer and the percentage saturation of haemoglobin

Position in molecule															
Residue No.															
Postulated sequence of ζ -chain															
Amino acids found in known α -chains															
	N	7	8	9	10	11	16	17	18	19	20	21	22	23	
	Terminus	(LYS)	THR	SER	LEU	LYS	(LYS)	ILE	THR	SER	ASN	ALA	THR	GLU	
		LYS	THR LYS (ala) (gly) (asn)	ASN (his) (ala)	VAL ILE	LYS	LYS	VAL ILE	GLY ALA (pro)	GLY ALA (pro) (ser)	HIS ASX (lys)	ALA	GLY ALA (glu) (asp)	GLU gly (asp)	
		I	I	E	E	I	I	$\alpha_1\beta_1$ contact	$\alpha_1\beta_1$ contact	I haem	I	$\alpha_1\beta_1$ contact	$\alpha_1\beta_1$ contact	$\alpha_1\beta_1$ contact	
		24	25	26	27	28	29	30	31	32	33	34	35	36	
		ILE	GLY	THR	GLU	THR	LEU	GLU	ARG	LEU	LEU	HIS	SER	PHE	
		TYR (ala) (ile)	GLY (val) (ala)	ALA (gly) (thr)	GLU	ALA (gly) (thr)	LEU (val)	GLU (asp) (gly)	ARG	MET (thr)	PHE (leu)	LEU (ala) (gln) (his) (thr) (ile)	SER (gly) (val)	PHE (tyr)	
		E $\alpha_1\beta_2$ contact	E $\alpha_1\beta_2$ contact	Haem	E deoxy $\alpha_1\beta_2$ contact	—	E	E	Haem 'Distal His'	I	E	E	—	E	E
		37	38	39	40	56	57	58	59	60	61	89	90		
		PRO	GLN	THR	LYS	(LYS)	ALA	HIS	GLY	SER	LYS	(LYS)	ILE		
		PRO	THR (gln)	THR	LYS	LYS (gln)	GLY (ala)	HIS	GLY	LYS (gln)	LYS	HIS (tyr) (ser)	LYS		
		E	E	CC	SC CC	I Haem	CC	CC $\alpha_1\beta_1$	I $\alpha_1\beta_1$	I	I $\alpha_1\beta_1$	I $\alpha_1\beta_1$	I $\alpha_1\beta_1$		
		91	92	99	100	101	102	103	104	105	106	107			
		LEU	ARG	(LYS)	ALA	LEU	SER	HIS	CYS	LEU	GLY	LYS			
		LEU	ARG	LYS	LEU (ile)	LEU	SER (gly) (ala)	HIS (glu) (asn)	CYS (ser) (his)	LEU (phe) (ile)	LEU (val)	VAL (ser)			
		I	I	I	SC $\alpha_1\beta_1$	E	SC	E $\alpha_1\beta_1$	CC	I	I	CC			
		108	109	110	111	112	113	114	127	128	129	130			
		GLN	LEU	ALA	SER	HIS	LEU	TYR	(LYS)	PHE	LEU	ALA			
		THR (val) (gly)	LEU (phe) (ile)	ALA (val) (met)	CYS ALA (val) (ser) (asn) (phe)	HIS (tyr)	LEU (his) (val)	PRO	LYS	PHE	LEU (phe)	ALA (ser) (thr) (cys) (gln)			
		CC	I	CC	CC	SC CC	I	CC	CC	E	$\alpha_1\beta_2$	E			
		131	132	133	134	135	136	137	138	139	140	141			
		SER	VAL	SER	GLN	VAL	LEU	THR	SER	LYS	TYR	ARG			
		SER ASN (ala)	VAL (leu)	SER (gly) (ala)	THR (leu)	VAL (ala)	LEU	THR (ser) (asx)	SER ala glu	LYS	TYR	ARG			

Fig. 1 In this figure the amino acid composition of peptides from the ζ -chain determined by Capp *et al.*⁸ are arranged to give a postulated sequence corresponding to that of the known α chains⁹. It can be seen that the fit is very good except at positions 90 and 114 which are external and position 107 in the $\alpha_1\beta_1$ contact. E, External; I, Internal; haem, haem contact; (LYS) in brackets in the postulated sequence assumed to be present from tryptic cleavages seen. Amino acids found in known α chains: Caps—common, lower case and brackets—rare. ↓, Tryptic cleavages. The positions of the various residues in the haemoglobin molecule are from the data of Perutz and his coworkers (for refs see ref. 7).

with oxygen were then calculated³. The use of the second sample position of the Unicam SP 1800 (SP 800(0)) recording spectrophotometers allows the direct recording of spectra of intracellular haemoglobin with minimal loss of precision as a result of light scattering.

Oxygen dissociation curves were obtained from the red cells from six embryos with a CR length of less than 35 mm. In these cells the embryonic haemoglobins amounted to between 30 and 50% of total haemoglobin. Similar studies were carried out on red cells obtained from several foetuses (Table 1). The oxygen affinities of the embryonic red cells do not differ from those of foetal red cells in the conditions used, the P50 (partial pressure of oxygen at 50% saturation of haemoglobin with oxygen), Bohr shift and haem-haem interactions being very similar. In order to exclude the presence of high or low affinity components the partial pressures of oxygen necessary for 20 and 80% saturation were also compared and found to be identical. These studies therefore show no difference in the oxygen dissociation properties of embryonic red cells compared with foetal red cells and further studies are necessary to elucidate the physiological significance of these haemoglobins.

Table 1 Comparison of the oxygen affinity of human embryonic* and foetal red cells suspended in isotonic phosphate buffers

Red cells suspended in isotonic buffer pH 7.13	Embryonic red cells (6 samples)	Foetal red cells
p20 mmHg	13-17	12-16
p50 mmHg	25-30	22-27
p80 mmHg	38-46	38-46
Haem-haem interactions (n value†)	about 2.5	about 2.6 normal adult red cells
In isotonic buffer pH 7.46	(4 samples)	
p20 mmHg	7.5-9.5	9-10.5
p50 mmHg	18-20	17.5-18
p80 mmHg	29-32	27.5-28
Bohr shift	-0.43 to -0.49	-0.42 to -0.52

*Cells from embryos with a crown-rump measurement of less than 40 mm and containing more than 30% embryonic haemoglobins.

†The slope of the line $\log y/(1-y)$ against $\log P_{O_2}$ where y is the fractional saturation with oxygen as a measure of the haem-haem interactions.

As haem-haem interactions are thought to require a pair of different chains (such as $\alpha_2\beta_2$) in each haemoglobin molecule and do not occur in haemoglobins consisting of one type of chain only (such as Hb- β_4 or Hb- γ_4), our finding that the haem-haem interactions of embryonic haemoglobins were normal argues against the presence of a major haemoglobin species containing one type of chain only, as previously suggested for Hb-Gower 1 (ref. 4). Studies of the embryonic haemoglobins have shown that there are two types of embryonic globin chains, ϵ -chains and ζ -chains. The ϵ -chain is found in Hb-Gower 2 in combination with α -chains, Hb- $\alpha_2\epsilon_2$. The other embryonic chain, the ζ -chain, is found in combination with γ -chains in Hb-Portland, $\zeta_2\gamma_2$ (ref. 5), and in combination with β -chains, Hb- $\zeta_2\beta_2$ (ref. 6), and it has been suggested that the ζ -chain is an α -type chain⁷. Comparison of the composition of some tryptic peptides from the ζ -chain with that of other mammalian haemoglobin α -chains (Fig. 1) confirms this idea. Previously it has been shown⁴ that Hb-Gower 1 contains ϵ -chains but not any α -chains. Our observation that the haem-haem interactions are the same in embryonic red cells as in foetal or adult red cells indicates that Hb-Gower 1, which forms a large haemoglobin component in the former, must contain α -like chains as well as ϵ -chains. It is, therefore, suggested that it consists of ζ -chains combined with ϵ -chains: Hb- $\zeta_2\epsilon_2$.

Note added in proof: The peptide described by Capp *et al.*⁸ from their ζ -chain preparation from Hb-Portland which could not be fitted to the α -chain sequence (Fig. 1) appears to correspond to the first part of the additional peptide found¹⁰ attached to the

C-terminal end of the α -chain in Hb-Icaria present in some cases of α -thalassaemia.

Residue no.	142	143	144	145
Hb Icaria	LYS	ALA	GLY	ALA
Postulated additional peptide from ζ -chain	LYS	ALA	GLY	ALA
Residue no.	146	147	148	149
Hb Icaria	SER	VAL	ALA	VAL
Postulated additional peptide from ζ -chain	SER	LEU	THR	THR

It is not clear however whether this peptide is present in all samples studied (that is, those from α -thalassaemia as well as those from normal embryos).

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Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth

PROGESTERONE has long been considered an antagonist of oestrogen action¹. The delicate balance and interactions between these ovarian hormones are essential for many reproductive functions. Early studies in chick oviducts and uteri of rats and mice have shown that the simultaneous administration of progesterone and oestrogen resulted in inhibition or modification of oestrogen-induced growth of these target organs²⁻⁶. One possible mechanism by which progesterone could be antagonistic to oestrogen is by suppressing the quantity of cytoplasmic oestrogen receptor. It is generally held that the mechanism by which oestrogen, O, stimulates uterine growth depends on the binding of O to cytoplasmic receptors, R_c, to form R_cO complexes⁶. These R_cO complexes are translocated to nuclear sites where they probably stimulate nuclear events that cause the uterus to grow⁷. Translocation and nuclear accumulation of receptor-oestrogen complexes, R_nO, are accompanied by a concomitant decrease in the quantity of R_c (ref. 8). During the period when R_c is reduced, the uterus is insensitive to additional exogenous oestrogen⁹. Gradually R_c is replenished by processes which may involve reutilisation and/or resynthesis⁸. Work from our laboratory indicates that the mechanism of action of non-steroidal oestrogen antagonists resides in their inability to stimulate the replenishment of the cytoplasmic oestrogen receptor, R_c, thereby rendering oestrogen responsive tissues less sensitive to oestrogen^{10,11}. The possibility that progesterone might also act as an oestrogen antagonist by reducing the amount of oestrogen R_c has been suggested^{12,13}. In this report, we demonstrate that progesterone does decrease the quantity of oestrogen receptors by interfering with the replenishment of R_c and that this decrease results in a reduced sensitivity of uterine tissue to oestrogen.

To establish the effect of progesterone on the quantity of oestrogen receptor, it is necessary to use assay techniques that permit the measurement and distribution of total receptor^{14,15}. The ³H-oestradiol exchange assay, as developed in this laboratory and elsewhere^{15,16} enables us to make such measurements.

Table 1 Effect of progesterone on various uterine growth parameters and concentration of cytoplasmic oestrogen receptor

Pretreatment	Uterus (day 4)		Cytoplasmic receptor (day 4)	
	Wet weight (mg $\pm$ s.e.m.)	Protein (mg $\pm$ s.e.m.)	DNA (μ g $\pm$ s.e.m.)	
<i>a</i> (OL)	92.6 $\pm$ 4.5	8.81 $\pm$ 0.41	679.7 $\pm$ 17.8	pmol per 100 mg protein
<i>b</i> (OL + P)	69.0 $\pm$ 2.3†	8.20 $\pm$ 0.57*	744.5 $\pm$ 26.1†	pmol per mgDNA

Immature rats were treated with hormones as described in Fig. 1 and uterine wet weight, protein and DNA content were determined on day 4 by the methods of Lowry *et al.*²⁴ and Ceriotti²⁵. The values represent the mean $\pm$ s.e.m. from six to eight experiments with six animals per experiment.

*No significant differences between *a* and *b*

†Significant differences ($P < 0.01$) between *a* and *b*

Immature female Sprague-Dawley rats (22 d old) were purchased from Texas Inbred Mice Co. and housed in a controlled environment with 14 h of artificial light between 0700 and 2100. All rats received two daily subcutaneous injections of 2.5 μ g oestradiol 17- β in 0.5 ml of saline containing 1% ethanol. The pretreatment with oestradiol was done to increase the sensitivity of the uterus for progesterone, presumably through the effect of oestrogen on increased synthesis of the progesterone receptor^{17,18}, and to maximise the translocation of

amount of cytoplasmic oestrogen receptor in the uterus on day 4 (24 h after the injection, Fig. 1 and Table 1). A reduction was also observed in the amount of R_nO present in the nuclear fraction although these quantities in either group are small and hence have little influence on the total amount of receptor (Fig. 1). Antagonistic effects of progesterone are also observed at this time by the reduced wet weight of the uterus (Table 1). But, the decrease in the quantity of R_c does not result from a general depressive action of progesterone on uterine protein synthesis, since the decrease can still be observed when the data are expressed as sites per mg protein (Table 1).

Since it has been shown that progesterone does not interfere with the ability of oestrogen to cause the translocation of the RO complex from the cytoplasm to the nucleus¹⁵, the reduced R_c 24 h after progesterone treatment probably results from the blockade of the replenishment process. To test this possibility the quantity of R_c was determined at various times after treatment with oestrogen (O), or oestrogen plus progesterone (O + P) (Fig. 2). These data show that the level of R_c is depleted immediately after injection of oestrogen plus progesterone and that replenishment occurs during the next 24 h. In the case of oestrogen plus progesterone, the level of R_c does not exceed the quantity present 24 h earlier. In contrast, oestrogen alone results in a significant increase above control level. This antagonistic influence of progesterone is dose-dependent and specific for progestational hormones (data to be reported elsewhere).

The reduction in the quantity of cytoplasmic oestrogen receptor following progesterone treatment should make the uterus less responsive to oestradiol. To assess this possibility animals were treated with oestradiol or oestradiol plus progesterone as described in Fig. 1 and on day 4 all animals were injected with 2.5 μ g of oestradiol. The wet weight, dry weight and protein content of the uterus were examined 24 h later. Oestrogen treatment increased wet and dry weight in both OL and OL + P pretreatment groups when compared with saline injected controls (Table 2). But the ability of oestradiol (OL) to increase uterine weight was significantly depressed ($P < 0.01$) in the OL + P pretreatment group when compared with OL pretreatment group (Table 2). This was also true for the protein content of the uterus (OL pretreatment group, 10.7 ± 0.2 mg per uterus; OL + P pretreatment group, 8.7 ± 0.3 mg per uterus). No appreciable difference among treatment groups was observed, however, for saline-injected controls. These data indicate that the antagonistic effect of progesterone is correlated with its depressive effect on the quantity of cytoplasmic

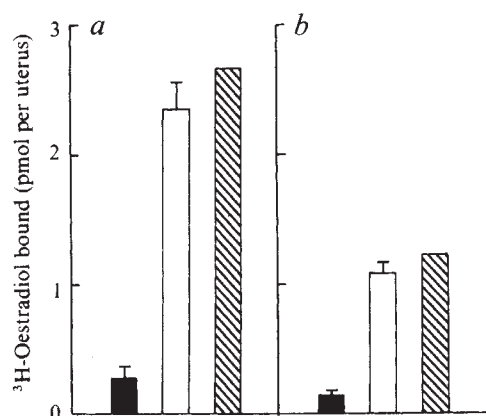


Fig. 1 The effect of progesterone on the quantity of oestrogen receptor in the rat uterus. Two groups of immature rats were given 2.5 μ g of oestradiol for 2 d as described in the text. Group *a* received another injection of oestradiol on day 3 while group *b* received oestradiol plus progesterone. On day 4 the quantity of oestrogen receptor was determined in the nuclear (■) and cytoplasmic (□) fractions as described in text. The total (///) amount of receptor (■ + □) is also shown. The data represent the mean $\pm$ s.e.m. from six to eight experiments with 6 animals per experiment.

the R_cO complex to the nucleus. On the third day rats were divided randomly into two groups. Animals in group A were injected with 2.5 μ g of oestradiol in 0.5 ml of saline and 0.2 ml of sesame oil. Animals in group B were injected with 2.5 μ g of oestradiol plus 2.5 mg of progesterone dissolved in 0.2 ml of sesame oil. In both groups, the sesame oil or progesterone in oil was injected at a separate subcutaneous site and did not affect the adsorption of oestradiol. On day 4 uteri were removed and analysed for cytoplasmic and nuclear receptor by the ³H-oestradiol exchange assay^{15,16}.

Progesterone treatment caused a significant reduction in the

Table 2 Antagonism of oestrogen uterine growth by progesterone

Pretreatment (day 3)	Treatment (day 4)	Uterine weight			
		Wet weight (mg $\pm$ s.e.m.)	% Saline control	Dry weight (mg $\pm$ s.e.m.)	% Saline control
<i>a</i> (OL)	OL	151.0 $\pm$ 20.0*	228	23.0 $\pm$ 1.3*	195
	S	66.1 $\pm$ 2.9†	100	11.8 $\pm$ 0.5†	100
<i>b</i> (OL + P)	OL	89.0 $\pm$ 4.0*	155	17.6 $\pm$ 0.6*	166
	S	57.3 $\pm$ 2.3†	100	10.6 $\pm$ 0.4†	100

Immature rats received hormone treatment as described in Fig. 1. On day 4 they were injected with 2.5 μ g of oestradiol (OL) or saline (S) and the uterine weight was determined on day 5. The values represent the mean $\pm$ s.e.m. of five experiments with six rats per experiment.

*Significant differences between pretreatment groups ($P < 0.01$).

†No significant differences between pretreatment groups.

oestrogen receptor. When considered in the light of the results from Figs 1 and 2, these data suggest that progesterone decreases cytoplasmic R_c and thereby reduces the sensitivity of the uterus to subsequent oestrogen action.

The antagonistic effects of progesterone on uterine weight and protein content probably reflect the ability of progesterone to redirect the manner in which certain uterine cell types will respond to oestrogen. Histological examination of the uterus on day 5 indicates considerable changes in the epithelial and stromal elements of the uterus (data not shown). In the OL+P pretreatment group, the height of the luminal epithelium is greatly reduced and the surface area of the lumen is expanded. In addition, the quantity of stroma seems to be reduced although the quantity of myometrium is only slightly affected. Thus, progesterone may reduce the amount of R_c in certain cell types and not in others, thereby differentially controlling the action of oestrogen.

Several reports have suggested that progesterone has a depressive effect on the cytoplasmic oestrogen receptor. The cytoplasmic fraction of human uterus binds more oestrogen during the follicular phase than during the luteal phase of the menstrual cycle^{19,20}. The ability of the rabbit uterus to bind oestrogen has also been shown to decrease during pseudopregnancy and

the ability of a tissue to respond to oestrogen, is basic to the elucidation of the progesterone-oestrogen interactions during various reproductive states.

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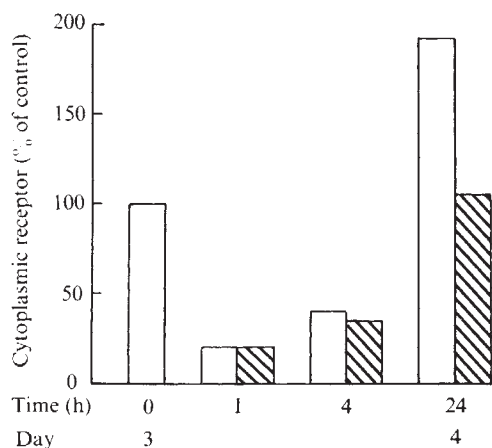


Fig. 2 The effect of progesterone on the oestrogen-induced depletion and replenishment of the cytoplasmic oestrogen receptor. Immature rats received 2.5 µg of oestradiol for 2 d and on the third day they were injected with either oestradiol (□) or plus oestradiol and progesterone (▨). The quantity of cytoplasmic oestrogen receptor was determined at 1, 4, and 24 h after injection. The values represent the mean of three experiments with four animals per experiment.

increase subsequent to luteotomy²¹. Thus, in conditions when the concentration of progesterone in the blood is high, the oestrogen binding capacity of the uterus is decreased. Brenner *et al.*¹³, made similar observations with the monkey oviduct. In the above studies indirect methods or methods that do not permit the evaluation of the quantity and distribution of total receptor were used. The methods we used permit this evaluation and demonstrate conclusively that progesterone can decrease the quantity of cytoplasmic oestrogen receptor. Also, this decreased quantity of R_c is correlated with a reduced or redirected ability of the uterus to respond to oestrogen.

It is known that oestrogens and progestins bind to separate cytoplasmic receptor molecules²² and that the receptor-steroid complexes thus formed do not compete for the same nuclear sites^{15,23}. Therefore, it seems that progesterone or the progesterone-receptor complex influences the replenishment of the cytoplasmic oestrogen receptor by interfering with its resynthesis or recycling, thereby reducing the quantity of oestrogen R_c in the uterus. This concept, that progesterone can reduce the quantity of oestrogen receptors and thereby reduce or modify

Immunoperoxidase staining of α -bungarotoxin binding sites in muscle endplates shows distribution of acetylcholine receptors

α -BUNGAROTOXIN (α BT) and similar toxins are specific ligands for the nicotinic acetylcholine (ACh) receptors in skeletal muscle^{1,2} fish electric organ³⁻⁵ and retina⁶ and other parts of the central nervous system^{7,8}. Fluorescent^{9,10} and autoradiographic^{11,12} methods have been used to visualise the α BT binding sites in muscle and electric organ. We report here an α BT-immunoperoxidase staining method for light and electron microscopy, which is relatively convenient and has high resolution. We have used this method to study the distribution of ACh receptors in skeletal muscle.

Mouse diaphragms or frog (*Rana pipiens*) sartorius muscles were agitated gently for 1.5–2 h with 5×10^{-8} M α BT in either F14¹³ tissue culture medium with 10% foetal calf serum at 37 °C in a 5% CO₂ atmosphere (mouse), or frog Ringer solution with 2 mg ml⁻¹ bovine serum albumin (BSA) at room temperature (frog). Controls were either incubated without α BT or preincubated for 10 min with 10^{-3} M decamethonium (a specific inhibitor of α BT binding) followed by 5×10^{-8} M α BT with 10^{-3} M decamethonium. The tissues were then washed extensively in incubation medium without α BT or decamethonium (the last washes without serum or BSA), fixed for 90 min at 0–4 °C in a solution containing 2% paraformaldehyde, 0.01 M NaIO₄, 0.075 M lysine and 0.037 M phosphate buffer, final pH about 6.9 (ref. 14), cut in pieces, washed in 0.1 M sodium phosphate buffer, pH 7.1 (PB), cryoprotected by soaking in 5% dimethylsulphoxide in PB, and quick frozen. For electron microscopy, cryostat sections 20 µm thick were fixed to gelatin-coated plastic dishes with vapours of

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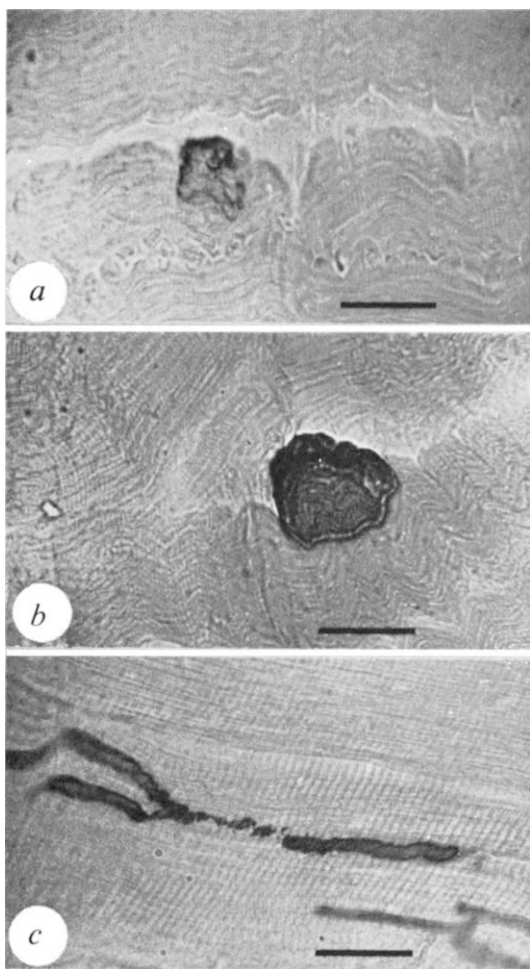


Fig. 1 *a*, α BT-immunoperoxidase-stained endplate in mouse diaphragm. *b*, Acetylcholinesterase-stained endplate in a parallel section. *c*, α BT-immunoperoxidase-stained endplate in frog sartorius muscle. Bright field photomicrographs. Reference bars represent 20 μ m.

8% paraformaldehyde at 40 °C, washed with PB, and treated at room temperature as follows: (1) 60 min in rabbit anti- α BT (ABT), 29 μ g ml⁻¹, in PB with 0.1 mg ml⁻¹ BSA (PB-BSA); (2) 30-min wash with PB-BSA; (3) 60 min in a horseradish peroxidase conjugate of the IgG fraction from goat antiserum to rabbit IgG (AR-HRP), 13 μ g ml⁻¹ in PB-BSA. (ABT was purified from rabbit antiserum to α BT by affinity chromatography on a Sepharose- α BT column. One milligram of ABT precipitated 22 μ g of α BT in a precipitin test. AR-HRP was made from the IgG fraction of goat antiserum to rabbit IgG (Miles) by conjugation with horseradish peroxidase (RZ-3.2, Worthington) according to the two-step method of Avrameas and Ternynck¹⁵, except that the conjugation step was done at pH 9.0. The conjugate had an A_{410}/A_{280} of 0.49, thus approximately a 1.2 molar ratio of peroxidase to IgG.) Antibody-treated sections were washed extensively with PB-BSA and PB, refixed in 2% glutaraldehyde in PB, washed, and stained for 30 min at room temperature with 3,3'-diaminobenzidine (DAB, 0.1 mg ml⁻¹) and 0.001% H₂O₂ in 0.05 M Tris-HCl, pH 7.6 (ref. 16), washed for up to 3 h, postfixed in 1% OsO₄ in PB for 45 min, then dehydrated and embedded in Epon. Except where stated, thin sections were not poststained with uranyl acetate or lead citrate. This omission preserved the high contrast between the DAB reaction product and the tissue background. For light microscopy, cryostat sections 12 μ m thick were dried on to glass slides and treated as above, omitting OsO₄ treatment, cleared and mounted under coverslips; alternate sections were stained for acetylcholinesterase¹⁷.

As seen by light microscopy (Fig. 1), the α BT-immunoperoxidase stain was limited to the muscle endplates. The observed patterns of staining resembled the characteristic paths of nerve endings in these species as delineated by silver or gold impregnation. In mouse diaphragm (Fig. 1*a* and *b*) the immunoperoxidase stain gave the endplate the appearance of convoluted strands but the cholinesterase stain was less restricted, filling the endplate region. In frog sartorius, however, both immunoperoxidase (Fig. 1*c*) and acetylcholinesterase stains followed the paths of the nerve endings. Omission of α BT or either antibody from the staining procedure completely eliminated endplate staining. Compounds known to interact specifically with nicotinic ACh receptors were assayed for their effects on α BT-immunoperoxidase staining. Cryostat sections of untreated mouse diaphragm 10 μ m thick were incubated for 10 min at room temperature with a 10⁻³ M concentration of one of the following: *d*-tubocurarine, carbamylcholine (with 10⁻³ M eserine), nicotine, or decamethonium, in phosphate buffered saline¹⁸ with BSA (2 mg ml⁻¹), and then incubated 30 min more with the addition of 5 $\times$ 10⁻⁷ or 5 $\times$ 10⁻⁸ M α BT. The sections were then washed, fixed, and immunoperoxidase stained as described above. All the ligands markedly reduced endplate staining at both concentrations of α BT and all were more effective at the lower concentration. Decamethonium was the most effective blocker, virtually eliminating endplate staining at 5 $\times$ 10⁻⁸ M α BT concentration. The ability of these ligands to block the staining reaction at the concentrations used is consistent with the known pharmacology of the binding of α BT and similar toxins to ACh receptors²⁻³. These pharmacological results, together with the immunochemical controls mentioned, show the α BT immunoperoxidase stain to be highly specific for nicotinic ACh receptors. Staining of the same specificity was also obtained in diaphragms fixed in paraformaldehyde before freezing, sectioning and incubation with α BT.

Electron microscopy (Fig. 2) confirmed the restriction of staining to the endplate (Fig. 2*a*) and revealed a non-homogeneous distribution of stain within the endplate (Fig. 2*b* and *f*). The heaviest staining was on the muscle plasma membrane at the 'tops' of the postsynaptic folds, with little or no stain at the 'bottoms' of the folds. Some of the stain was on the presynaptic plasma membranes. This was less intense than the postsynaptic stain. A qualitatively similar distribution of stain was seen in the diaphragm of a mouse killed by intravenous injection with 10 μ g of α BT. Staining was almost eliminated by the presence of 10⁻³ M decamethonium during α BT incubation (Fig. 2*d*) and completely eliminated by omission of α BT (Fig. 2*c*).

The presence of some presynaptic ACh receptors cannot yet be ruled out by this method; however, it seems that either specific antigen-antibody complex or DAB reaction product originally attached to postsynaptic ACh receptors was translocated and later fixed to the presynaptic membranes. This was most clearly shown by the presence of stain on any structure located directly opposite a site of strong postsynaptic membrane staining. These structures included Schwann cell processes (Fig. 2*b* and *f*), and the muscle basement membranes in the synaptic cleft (Fig. 2*f*) or in denervated muscle (A. N. Bender, S. P. Ringel, B. W. Festoff, W. K. Engel, Z. V. and M. P. D., unpublished), as well as the presynaptic nerve membrane. This spreading of stain could also account for the staining of the membranes in the relatively shallow folds of the frog endplates (Fig. 2*f*).

It is clear that most, if not all, of the muscle ACh receptors are restricted to that region of the postsynaptic plasma membrane which is nearest to the nerve ending and is contiguous with the postsynaptic dense material normally seen in thin sections poststained with uranyl acetate and lead citrate (Fig. 2*e*). This is in agreement with the results of electron microscope autoradiography with radioactive

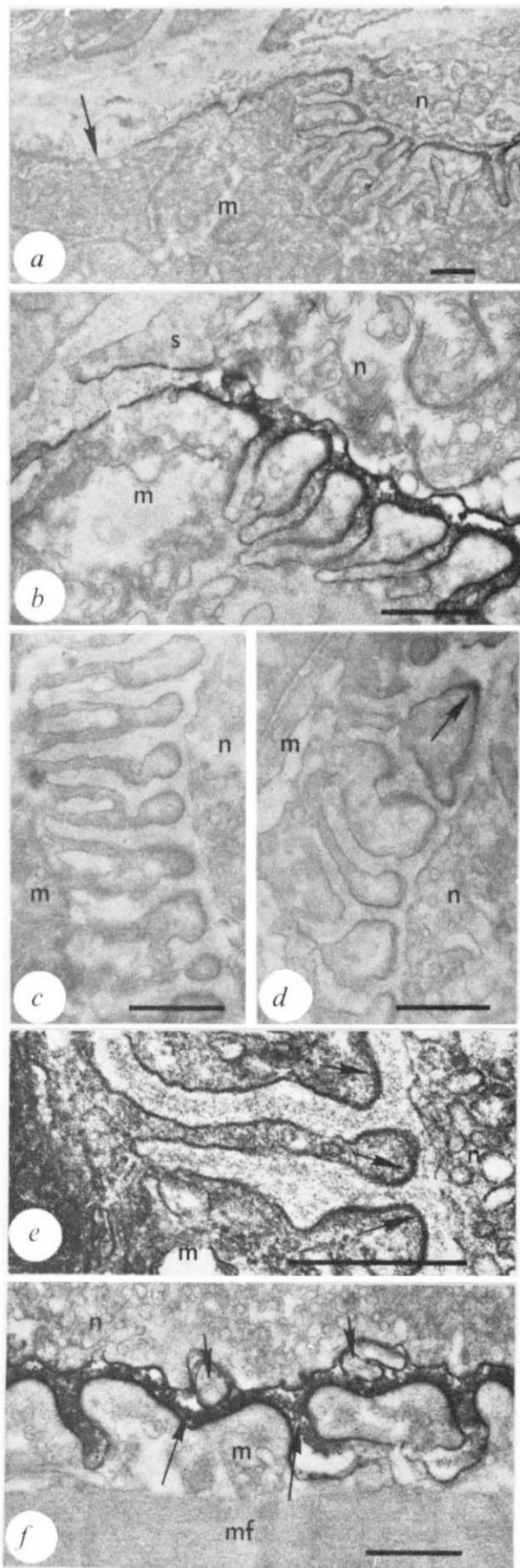


Fig. 2 Mouse diaphragm (a-e) and frog sartorius muscle (f) endplates stained by the α BT-immunoperoxidase method after various treatments. a, α BT-treated. The endplate is stained, but the nearby muscle plasma membrane (arrow) and other structures are not. b, α BT-treated. Most of the stain is on the muscle plasma membrane at the tops of the postsynaptic folds. There is some stain in the synaptic cleft and on the nerve and Schwann cell plasma membranes. c, Control incubated without α BT. There are no stained structures. d, Decamethonium and α BT-treated. There is little or no stain (arrow) on the muscle and nerve plasma membranes. e, Control incubated without α BT. This thin section was poststained with uranyl acetate and lead citrate. Note the post-synaptic dense material (arrows). f, α BT-treated (frog sartorius). The staining pattern resembles that in mouse. Note the staining of Schwann cell processes (short arrows) and of the basement membrane (long arrows). Reference bars represent 0.5 μ m. n, Nerve ending; m, muscle; mf, myofibril; s, Schwann cell process.

α BT¹². The postsynaptic distribution of ACh receptors revealed by the α BT-immunoperoxidase method also corresponds to that of the 80–120-Å membrane particles observed in freeze-fractured^{19–20} and in thin-sectioned²¹ muscle.

The α BT-immunoperoxidase method offers high resolution of the ultrastructural distribution of nicotinic ACh receptors and should be useful for light and electron microscopic evaluation of the distribution of ACh receptors in the central nervous system where structural and functional complexity pose serious obstacles to neurophysiological methods of receptor analysis. Our preliminary results with chicken retina indicate that the method will indeed be useful in examining the central nervous system.

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Putative neurotransmitters in clonal cell lines

THE regional distribution of putative neurotransmitters in the mammalian central nervous system (CNS) has been studied often (for example, refs 1–4). Although in some cases neurotransmitter synthesis has been correlated with nervous function, its distribution among different cell types has not been assayed. For example, the distribution of the putative neurotransmitters taurine and γ -aminobutyric acid (GABA) between nerve and glia has not been established using *in vivo* preparations. Another approach is to use clonal cell lines derived from the CNS, for their cellular homogeneity facilitates the assay of pool sizes of neurotransmitters and free amino acids in identified cells. Such determinations have been made with fibroblasts^{5–7}.

The expanding collection of clonal cell lines from the CNS⁸⁻¹¹ and other tissues (for example, refs 12-14) presents the opportunity to extend this approach. We describe here the free amino acid pools of some cell lines from the CNS and other tissues which exhibit differentiated phenotypes. We have found that although several specific differences exist between cells from different tissues, there is a strong similarity between different cell types of common embryological origin.

We used the rat nerve cell lines B35, B50, B65, B103 and B104, and the rat putative glial cell lines B11, B12, B15, B23, B28, B49, B82, B90, B92 and B108 (ref. 11), and two other lines of glial origin, C6 (ref. 8) and RN2 (ref. 15). Additional clonal cell lines examined were: smooth muscles BC₃H1 (ref. 16) and A10 (B. Kimes and B. Brandt, submitted); skeletal muscle L6 (ref. 13); plasmacytoma C1 and lymphomas S1A and S49 (ref. 14); neuroblastoma clones C1 (ref. 10) and N18 (ref. 17); melanomas¹⁸ B78 (non-pigmented) and B559 (pigmented); hepatoma H35 (ref. 19); growth hormone and prolactin-producing pituitary cells GH₃ (ref. 20); adrenal cortical line Y1 (ref. 12), and the fibroblast cell line 3T3 (ref. 21). All cells were grown in modified Eagle's MEM containing 10% foetal calf serum¹¹.

Early stationary phase cultures, 2 or 3 d after cessation of net cell division, were used exclusively for the determination of free amino acids, for cells at this stage of the growth cycle usually express maximum morphological and biochemical differentiation^{22,23}. Cell viability was greater than 95% as measured by the uptake of fluorescein dibutyrate²⁴.

Free amino acids were extracted from cells washed four times in phosphate buffered saline (PBS, 150 mM sodium chloride, 10 mM sodium phosphate, pH 7.3, 2 mM calcium chloride, 0.5 mM magnesium chloride) according to a modification of the trichloroacetic acid (TCA) procedure of Saifer²⁵. Frozen cell

pellets were homogenised at 0 °C with 20 volumes of 6% TCA using a Duall tissue grinder and centrifuged at 12,000g for 20 min. The procedure was repeated on the TCA precipitate, and the two supernatants were combined. A third extraction of the TCA precipitate yielded no more detectable free amino acids (less than 3% of the combined previous two extracts). The TCA was then extracted four times with 3 volumes of ethyl ether, the aqueous extract was lyophilised and dissolved in the starting buffer for the amino acid analysis. Amino acids were analysed with a Beckman amino acid analyser, Model 121, equipped with an automatic integrating device and run with an expanded elution profile²⁶. More than 40 amino acids and related compounds are resolved by this procedure. Since cell volume and protein per cell varies with chromosome number and growth phase (for example, ref. 27), the data are expressed as residues of a particular molecule per 1,000 residues of resolved free amino acids and related compounds.

GABA, glutamic acid, glycine, aspartic acid, taurine, (reviewed, for example in refs 28-30) and β -alanine³¹ are putative neurotransmitters in the CNS. Although little is known about their relative distribution between nerve, glia and other differentiated cells, clonal cell lines can be used to study their synthesis and/or accumulation. Table 1 shows the relative concentrations of the putative CNS transmitters in several cell types. There is little statistical difference between the GABA content of nerve and glial cells, although individual nerve lines contain much more GABA than observed in any glial line. The former is also true with the other putative neurotransmitters. The GABA pools do not necessarily correlate with glutamic acid decarboxylase activities^{11,23}, for the retention of amino acids was assayed, not synthetic ability. The only qualitative difference between cell types was the lack of detectable GABA and β -alanine in muscle and fibroblast cells.

Table 1 Relative concentrations of putative CNS neurotransmitters in clonal cell lines

Cell line	Cell type	Relative concentration (residues per 1,000 free amino acids $\pm$ s.d.)					
		GABA	β -ALA	GLU	ASP and ASPN	GLY	TAU
B35	Nerve	7.1 $\pm$ 0.3 (3)	35 $\pm$ 6 (3)	203 $\pm$ 6.5 (3)	53 $\pm$ 5 (3)	235 $\pm$ 17 (3)	207 $\pm$ 19.7 (3)
B50	Nerve	30.0	75.2	153	7.6	283	202
B65	Nerve	75.1 $\pm$ 13.1 (5)	51.6 $\pm$ 4.2 (5)	144 $\pm$ 10.1 (5)	21 $\pm$ 0.7 (5)	203 $\pm$ 7.8 (5)	123 $\pm$ 9.2 (5)
B103	Nerve	7.3 $\pm$ 0.1 (3)	20.6 $\pm$ 2.7 (3)	104 $\pm$ 6.5 (3)	46 $\pm$ 2.9 (3)	313 $\pm$ 7.5 (3)	96.1 $\pm$ 11.8 (3)
B104	Nerve	5.9	11.2	143	56.7	108	86.1
N18	Nerve	9.0	13.8	114	42.7	269	157
Nb-C1	Nerve	4.2	8.6	28.4	20.3	265	126
B11	Glial	13.4	35.8	171	49.7	144	190
B12	Glial	2.1	21.1	155	57.5	135	70.6
B15	Glial	1.5	9.3	103	105	96.2	67.5
B23	Glial	5.6	93.2	274	50.4	112	168
B28	Glial	19.5	48.1	212	52.7	153	240
B49	Glial	4.8	50.6	148	33.4	96.5	184
B82	Glial	1.1	2.3	84.0	85.7	90.7	28.8
B90	Glial	1.4	10.2	194	58.3	119	117
B92	Glial	2.1	4.0	178	69.2	65.9	22.9
B108	Glial	3.4	20.0	217	66.6	154	146
C6	Glial	15.5	3.3	309	65.4	163	30.9
RN2	Glial	4.4	38.7	156	20.1	227	127
B78	Melanocyte	6.1	26.4	48.7	18.6	329	71.8
B559	Melanocyte	3.5	5.5	50.5	54.9	195	41.3
S1A	Lymphocyte	1.4	30.1	172	25.7	281	209
C1	Plasmacyte	1.7	10.1	295	74.9	134	154
S49	Lymphocyte	1.7	14.2	198	34.2	250	152
Y1	Adrenal cortical	6.5	7.9	58.2	64.2	188	85.4
GH ₃	Pituitary	7.2	4.7	184	37.1	166	160
L6	Skeletal muscle	<0.1	<0.1	120	65.8	123	50.5
BC ₃ H1	Smooth muscle	<0.1	<0.1	64.0	81.3	99.2	137
A10	Smooth muscle	<0.1	<0.1	19.8	59	63	13.5
3T3	Fibroblast	<0.1	<0.1	59.0	78.5	82.6	68.6
H35	Hepatocyte	<0.1	<0.1	80.9	72.6	240	38.8
Serum		<0.1	3.5	80.6	60.5	59.3	16.7
Medium		<0.1	<0.1	<0.1	<0.1	8.4	<0.1

Free amino acids were extracted from cells by TCA precipitation and chromatographed on an amino acid analyser. Serum and medium samples were treated in an identical manner. The sum of the aspartic acid and asparagine peaks are given. Where indicated, the data are presented as the mean $\pm$ (s.d.) for *n* determinations using different cell preparations. Abbreviations: GABA, γ -aminobutyric acid; β -ALA, β -alanine; GLU, glutamic acid; ASP, aspartic acid; ASPN, asparagine; GLY, glycine; TAU, taurine.

Of the remaining amino acids, there were no significant differences between the different cell types. Finally, several unidentified, ninhydrin-reacting compounds eluted at times which did not correspond to the standards. For example, cells contained compounds which eluted between DL-O-phosphoserine and O-phosphoethanolamine, and between urea and hydroxyproline. These compounds never individually constituted more than a few residues per thousand, and were found in similar amounts in all cells.

The relative concentrations of free amino acids described in the nerve and glial cell lines are in general agreement with those published for whole brain (for example, ref. 25), although there are exceptions. For example, the concentration of glutamic acid was the highest of the free amino acids in whole cerebral tissue²⁵ and the nerve and glial cell lines (Table 1), but the concentration of aspartic acid was significantly higher in the brain than the cell lines (Table 1). Such discrepancies are, however, to be expected since the multiplicity of cell types found *in vivo* may not have been represented among the clonal cell lines.

Table 1 shows that cells derived from neuroectoderm contained detectable amounts of GABA and β -alanine, although the mesodermally derived muscle and fibroblast cells did not. Thus nerve, glia, melanocytes and neuroblastoma clones had qualitatively similar free amino acid pools distinct from those of muscle and fibroblast cells. It has been shown that nerve and glial cells share a number of additional traits distinct from muscle, such as acetylcholine and catecholamine synthesis, GABA uptake, S100 and 14-3-2 protein synthesis, and a morphological response to cyclic nucleotides^{11,32,33}. Other cell types, such as those derived from lymphoid tissue, the adrenal cortex and the anterior pituitary, also contained both GABA and β -alanine (Table 1). Thus GABA and β -alanine were excluded from most mesodermal tissues as opposed to being uniquely associated with nervous tissue. The apparent exception was the adrenal cortical clone, which is thought to be of mesodermal origin, but contained GABA (Table 1). The functional similarity between cells of the immune and nervous systems has, however, been pointed out repeatedly (for example, ref. 34). In addition, GABA has been found in other tissues in smaller amounts than in the nervous system and may serve a metabolic role (for example, ref. 35). Since no GABA was detected in the culture medium, cells containing GABA probably synthesise it. Although it is likely that these cells also synthesise β -alanine, this is not proven, for serum contained this compound (Table 1) and it may be concentrated. It should be noted, however, that cells contain both GABA and β -alanine, or neither, probably because glutamic acid decarboxylase can use both aspartic and glutamic acids as substrates to produce β -alanine and GABA, respectively³⁶.

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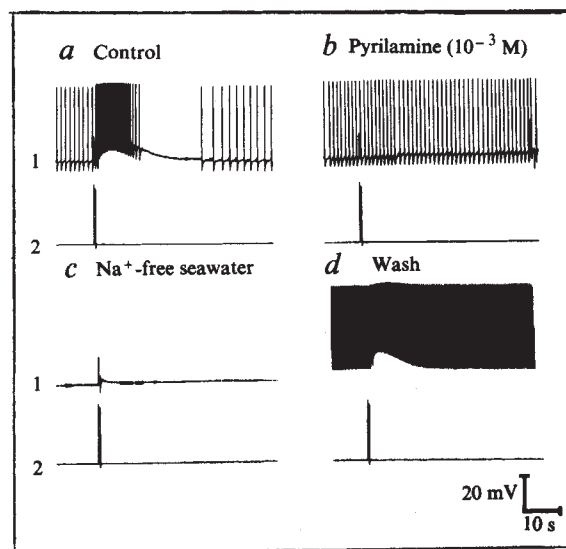
H₁ and H₂ histamine receptors on *Aplysia* neurones

WE have found two types of histamine receptor on neurones of the mollusc *Aplysia* corresponding to those already known in mammals. The presence of these specific receptors is consistent with the suggestion that histamine acts as a neurotransmitter.

Histamine, originating primarily from mast cells, is an important pharmacological agent in the regulation of smooth muscle and visceral functions in mammals¹. Two types of histamine receptor, H₁ and H₂, have been described. Activation of H₁ results principally in smooth muscle contraction and is blocked by pyrilamine and several other compounds. H₂ mediates stimulation of gastric secretion, increases in heart rate and inhibition of uterine contraction, and its response is blocked by burimamide but is insensitive to H₁ blocking drugs^{2,3}.

Although it seems likely that histamine functions as a neurotransmitter in the central nervous system (CNS), there is no definitive evidence for this. Some cortical neurones of the cat respond to iontophoretic histamine by both depression and excitation⁴, and a relatively high proportion of hypothalamic

Fig. 1 Responses of an unidentified neurone from the left pleural ganglion to 1,000-nC pulses of histamine. Trace 1 is the intracellular recording and trace 2 indicates the iontophoretic pulse. Pylamine was dissolved in seawater and perfused over the ganglion. Na⁺ replacement was made with Tris⁺ as previously described¹⁴.



neurones is excited by histamine⁵. It has been suggested, on the basis of postdegenerative changes in local histamine concentrations, that histaminergic pathways run through the medial forebrain bundle⁶. Histamine affects sympathetic ganglia, but it is not clear whether it functions in this system as a neurotransmitter or simply modulates other transmitter systems^{7,8}.

Previous work on an invertebrate (*Helix*) nervous system has demonstrated that both excitatory and inhibitory responses can be recorded when histamine is added to the bath and that both kinds of response are blocked by pyrilamine⁹. We have extended these observations, applying the drug iontophoretically, testing both H₁ and H₂ blocking drugs and determining which ionic conductances are changed by histamine.

Histamine is present in most identified neurones of *Aplysia* in concentrations of 10⁻⁶–10⁻⁵ M (ref. 10). Weinreich *et al.*¹¹ have found two small neurones in the cerebral ganglion containing histamine in concentrations as great as 4 × 10⁻⁴ M. While surveying for the presence of receptors for several amines, we have found two types of response to histamine, the pharmacological sensitivities of which correspond to those of mammalian H₁ and H₂ receptors.

Experiments were performed on pleural, pedal, cerebral and abdominal ganglia of *Aplysia californica* removed from the animal and maintained under flowing artificial seawater in a Lucite chamber. As before¹², neurones were penetrated with double-barrelled glass pipettes for recording and electrical stimulation. Putative neurotransmitters were applied iontophoretically, through a five-barrelled glass pipette controlled by an electronic unit which delivered constant charge rather than current. Histamine was placed in one of the barrels at a concentration of 1 M, pH 3.5.

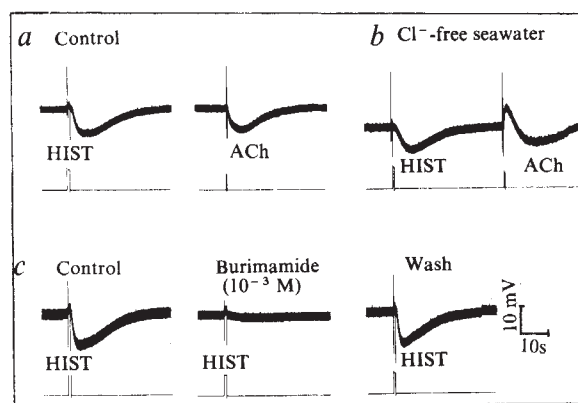


Fig. 2 Response of an unidentified cerebral neurone to histamine (HIST) and acetylcholine (ACh), 500-nC pulses. For (b) the preparation was perfused with a solution in which all Cl⁻ salts were replaced by isosmotic acetate salts. Burimamide was dissolved in seawater and perfused over the preparation.

Receptors for histamine were found on only a small fraction of neurones, and when present appeared both on the cell body and in the neuropile. All receptors were specific for histamine and showed no sensitivity to acetylcholine, dopamine, octopamine, phenylethanolamine or noradrenaline. Figure 1 shows the response of an unidentified neurone in the left pleural ganglion to a 1,000-nC pulse of histamine. After a very short latency, histamine caused a marked depolarisation and increase in discharge. The threshold for response was 500 nC. Pyrilamine (10⁻³ M), an H₁ blocking agent, reversibly abolished the response after exposure for 10 min (Fig. 1b). After recovery to control, the ganglion was perfused with seawater in which all Na⁺ was replaced with Tris⁺, which is at least relatively impermeable in this preparation. As Fig. 1c shows, the response was totally blocked in Tris⁺ seawater. This and all other responses to histamine which we have observed have been associated with an increase in

membrane conductance. Because both the voltage shift and the conductance change were absent in Na⁺-free seawater, this response seems to be the result of an increase in only Na⁺ conductance. The absence of action potentials in Fig. 1c results from an independent Na⁺ requirement. All these manipulations were reversible (Fig. 1d). This response was unaffected by exposure to 10⁻³ M burimamide for 20 min.

Figure 2 is from studies on an unidentified cerebral neurone with a different type of response. Figure 2a shows that the neurone responded with a slow hyperpolarisation to 500-nC pulses of both histamine and acetylcholine. An increased membrane conductance was measured during both responses. When all Cl⁻ in the seawater was replaced by the impermeant anion acetate, the membrane potential increased by about 7 mV and, whereas the histamine response remained essentially unchanged, the acetylcholine response became biphasic. The biphasic response resulted because acetylcholine caused an early increase in conductance of Cl⁻ followed by a late increase in K⁺ conductance¹³. The Cl⁻ component was depolarising in acetate seawater because the Cl⁻ equilibrium potential was then more depolarised. Because the histamine response did not change in acetate seawater, we assume that it, like the late acetylcholine response, resulted from a conductance increase to K⁺. Burimamide, the H₂ blocker, reversibly abolished this response in less than 5 min, but pyrilamine (10⁻³ M) had no effect after exposure for 30-min.

All histamine responses recorded were sensitive to either pyrilamine or burimamide, but not both. Both depolarising and hyperpolarising responses were found sensitive to each drug. These observations suggest that in *Aplysia* there are two types of histamine receptor corresponding to the H₁ and H₂ receptors described for mammals^{2,3}, and that each receptor is coupled to either an Na⁺ or a K⁺ conductance mechanism. Although identification of a substance as a neurotransmitter is absolutely dependent on demonstration of its release on nerve stimulation, and this has not been shown for histamine in this population of neurones, the presence of sensitive and specific receptors is consistent with a neurotransmitter function of histamine.

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Evidence for neuronal control of ion transport in chironomid larvae

THE role of anal papillae in the uptake of sodium and chloride ions has been established in the closely related culicid and chironomid larvae^{1–6}, but studies on the ultrastructure of these organs have been limited to the former group^{7–9}. The sites of active ion transport are located on the apical membrane of the epidermal cells below the cuticle^{5,8,10}. The mechanism by which ion transport is controlled in culicid larvae is most

probably hormonal^{4,11} but there are indications that a different system operates in the chironomid larvae¹²⁻¹⁴. To investigate this possibility, the ultrastructure of the anal papillae of *Chironomus riparius* Meigen has been examined. Axons were clearly present suggesting that ion transport may be under neuronal control.

The basic structure of the anal papillae of larval *C. riparius* is very similar to that described in mosquito larvae (Fig. 1). The cuticle is thin, relative to that over much of the rest of the body, and its surface is thrown into complex waves. The apical membrane of the epidermal cells is deeply infolded and numerous mitochondria are located adjacent to the proximal limit of these folds. The basal membrane is also irregular, leaving channels penetrating almost to the limit of the apical membrane. A number of 'mitochondrial pumps' similar to those described by Copeland⁷ in the corresponding region of *Culex quinquefasciatus* are situated among the intrusions of the basal membrane.

Two features distinguish the anal papillae of this chironomid species from those of mosquitoes. Tracheae and tracheoles are absent, but groups of axons are present in both the dorsal and ventral papillae of the chironomid. No axons have been reported in this region of any mosquito.

The examination of entire papillae, whether removed from the animal or not, did not reveal the presence of any nervous tissue. Similarly, conventional sectioning of wax-embedded papillae did not show any detail of the nerves. The use of silver staining on whole or sectioned tissue was also fruitless as the high concentration of Cl^- resulted in deposition of silver throughout the papilla¹⁵. The identification of axons has therefore been based exclusively on their appearance in thin sections examined in the electron microscope, where they are seen to contain microtubules, the diameter of which (about 20 nm) is compatible with that of typical neurotubules¹⁶ (Fig. 1).

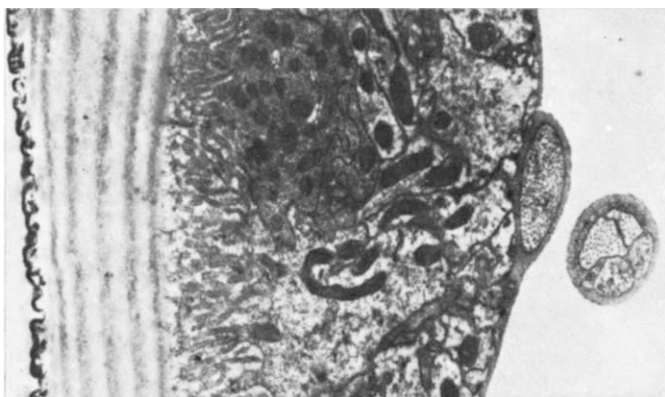


Fig. 1 The integument of the anal papilla of a larva of *C. riparius*, seen in thin section stained with uranyl acetate and lead citrate. Note the irregular membranes and small nerve. Distally, the individual axon in contact with the epidermis becomes invested in folds of the basal membrane. ($\times 9,350$)

The number of axons in each papilla is variable, but when sectioned transversely at their proximal end the number in each can be estimated. At this level there are usually twelve to fourteen axons in each of the ventral papillae and seven to ten in each dorsal papilla. As the degree of branching is unknown, closer estimates have not been attempted. The axons are grouped into two small nerves, diametrically opposed across the papilla, with occasional isolated axons separated from the main bundles. At this level in the papilla the axons are not always in contact with the epidermis. The source of the axons is not yet known.

Distally the number of axons or their branches is smaller, and individual axons can be seen penetrating the epidermis. They never seem to become intracellular but become closely

invested in folds of the basal membrane. Terminally, the axons increase in diameter and a swelling is formed that contains numerous vesicles, the diameter of which has been calculated as 80 ± 3 nm (ref. 17). The swelling is extracellular but located at the distal limit of the basal membrane near the mitochondria and apical membrane. No sense organs or cell bodies have been seen associated with these axons in the anal papillae and it is therefore presumed that they do not serve a sensory role.

The function of the axons and any transmitter which may be released from the vesicles at their termination is not yet known. If their existence, however, is related to Koch's observation that anticholinesterases inhibit net sodium transport¹², it may be concluded that the axons serve some role in the control of active ion uptake in the papillae of *Chironomus* larvae. As no axons have been reported in the papillae of mosquito larvae (nor could they be seen on examination of the published evidence) this conclusion would not contradict the idea of hormonal regulation of this activity in the mosquito *Aedes aegypti*¹¹. Nevertheless, it would emphasise that parallels between ionic regulation in culicid and chironomid larvae should be drawn with great care. Certainly, there may be differences in the sodium transporting mechanisms located in the anal papillae of these groups.

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Biosynthesis of γ -aminobutyric acid by isolated axons of cone horizontal cells in the goldfish retina

SPECIFIC proteolytic enzymes have been used to dissociate adult vertebrate retinæ into single cells¹⁻³. These methods facilitate the study of chemical and physiological properties of isolated, identified retinal cells. In particular, the synthesis of neurotransmitter candidates in a pure retinal cell type has been studied by drawing identified cells into a micropipette, incubating them with appropriate radioactive precursors, and analysing the presence of radioactive transmitters¹. In the study reported here, this method was used to examine transmitter synthesis by horizontal cells. Cajal's internal horizontal cells⁴ from the goldfish retina were used because they could be dissociated easily from the retina and identified readily. In addition, these cells have been shown to be connected to Cajal's external horizontal cells, and can therefore be considered as fusiform axon terminals of cone horizontal cells^{5,6}.

Before studying transmitters in dissociated cells, transmitter candidates synthesised by the whole goldfish retina were examined by the method of Hildebrand *et al.*⁷. As

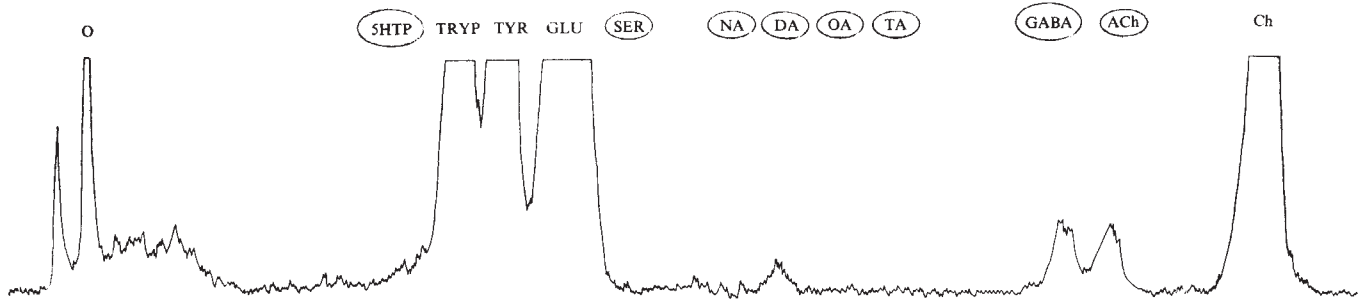


Fig. 1 Radiochemical scanning of an electrophoretogram from a homogenate of goldfish retina incubated with ^{14}C -labelled tryptophan, tyrosine, glutamic acid and choline chloride for 1 h. Two retinæ (about 100 mg tissue weight) from an adult goldfish (*Carissius auratus*), were isolated and incubated with 1.0 ml of isotonic Leibovitz medium⁸ (L-15, Grand Island Biological Co.) containing 10 μCi of each of methyl- ^{14}C -choline chloride (30 Ci mol⁻¹, Ch), L-U- ^{14}C -glutamic acid (255 Ci mol⁻¹, Glu), L-U- ^{14}C -tyrosine (54 Ci mol⁻¹, Tyr) and L-methylene- ^{14}C -tryptophan^{7,9} (54.5 Ci mol⁻¹, Tryp). After 0.5 to 2.0 h of incubation at 24 °C, the retinæ were homogenised with twice the tissue weight of formic acid (0.47 M)–acetic acid (1.4 M), and the homogenate was analysed by high voltage electrophoresis (6,000 V, 2 h) for the synthesis of ^{14}C -ACh, GABA, DA, noradrenaline (NA), octopamine (OA), tyranine (TA), 5-hydroxytryptophan (5-HTP) and serotonin (SER). Scale: origin (O) to Ch is 72 cm.

Fig. 1 shows, of all the transmitter candidates examined in this study, goldfish retinæ synthesise only acetylcholine (ACh), γ -aminobutyric acid (GABA) and dopamine (DA). ACh and GABA synthesised were identified by specific enzymatic degradation^{5,10} and ascending paper chromatography^{11,12}. DA was also identified by paper chromatography¹¹. Furthermore, significant activities of the

transmitter-synthesising enzymes choline acetyltransferase (EC 2. 3. 1. 6), glutamate decarboxylase (EC 4. 1. 1. 15), and tyrosine hydroxylase (EC 1. 14. 3a) in cell-free extracts of goldfish retina can be demonstrated (ref. 10 and D.M.K.L. unpublished).

To examine transmitter candidates synthesised by fusiform axons of cone horizontal cells⁶ (Cajal's internal horizontal cells⁴) from the goldfish retina, a pure population of these axons was prepared by a modified cell dissociation and separation method¹. By comparing the dimensions of the dissociated cells (Fig. 2b) with Golgi-impregnated fusiform axons of cone horizontal cells from the goldfish retina (Fig. 2a), isolated fusiform axons could be recognised easily and drawn into a glass micropipette individually (Fig. 2c and d) under visual observation. Figure 2 also shows that the isolated fusiform axons did not have nuclei, a finding consistent with earlier histological observations¹³.

A hundred isolated fusiform axons were drawn into a micropipette, incubated with H^3 -labelled precursors and analysed for transmitter synthesis. Results of analyses from a typical experiment showed that these fusiform axons synthesised and accumulated GABA, but no detectable ACh and DA (Fig. 3).

These results did not show whether the level of GABA synthesis by these axons was significant compared with that in the whole retina. To obtain this information the enzymatic activity of glutamate decarboxylase in extracts of these axons was measured. A hundred fusiform axons were drawn into a micropipette and lysed by repeated freezing and thawing with dry ice. Both the glutamate decarboxylase and choline acetyltransferase activities in isolated axons and whole retinæ were measured as described earlier^{1,9,10}. After a 3 h incubation, radioactive products were separated and identified by two-dimensional paper electrophoresis and chromatography¹¹. To calculate specific enzymatic activity, it is necessary to know the tissue weight of protein content. From microscopic measurements, the isolated fusiform axons used in this study had an average length of 100–200 μm and diameter of 3–6 μm . The specific activity of glutamate decarboxylase in these axons was calculated by assuming that an axon had a volume similar to a cylinder of radius 1.7 μm and 100 μm long, giving an approximate volume of 9×10^{-8} ml or a weight of 9.4×10^{-8} g per axon. On this basis the specific activity of glutamate decarboxylase in the isolated fusiform axons was 1.2 ± 0.4 μmol GABA formed $\text{h}^{-1} \text{g}^{-1}$ wet weight, a value similar to that measured in the whole retina (1.0 ± 0.1 , ref. 10). The specific activity of choline acetyltransferase in isolated fusiform axons (<0.05 μmol ACh formed $\text{h}^{-1} \text{g}^{-1}$ wet weight) was below the sensitivity of this assay and was less than 5% of that

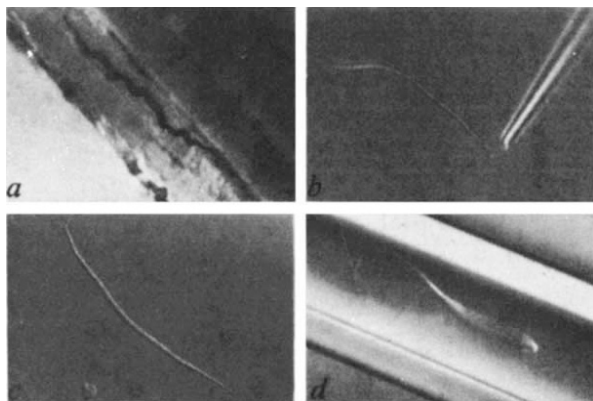


Fig. 2 a, A Golgi-impregnated fusiform axon (Cajal's internal horizontal cell) from the goldfish retina; b, an isolated fusiform axon from the goldfish retina; c, a fusiform axon drawn into a micropipette; d, a fusiform axon in a micropipette during incubation. ($\times 175$, Nomarski interference optics). The medium used for cell dissociation was modified L-15, made up of regular L-15 medium supplemented with glucose (2 g l⁻¹), ethyleneglycol bis(aminoethyl) tetraacetic acid (EGTA, 5 mM), penicillin (1,000 U ml⁻¹), streptomycin sulphate (0.5 mg ml⁻¹) and diluted to a tonicity of 250 mosmol, pH 7.2. In each experiment two to four goldfish retinæ (100–200 mg) were incubated at 24 °C for 2 h with gentle shaking in 3.0 ml of sterile, oxygenated modified, L-15 medium containing crystalline papain (EC 3. 4. 4. 10) (1.0 mg ml⁻¹, 11.2 U mg⁻¹, Sigma). Retinæ were then washed for 3 min with 5.0 ml modified L-15 medium and transferred to a conical tube containing 3.0 ml modified L-15 medium supplemented with 0.5% bovine serum albumin (fraction V, Sigma). Cells were dissociated by gently stirring and pipetting retinæ up and down through a wide-bore Pasteur pipette. After the suspension had settled for 5 min, 2.0 ml was taken from the top fraction and filtered through a sterile gauze pad to remove cell clumps. The filtrate was then applied to a 40-ml velocity sedimentation chamber (made from a 50-ml plastic syringe) for cell separation¹. Cells were separated by unit gravity at 24 °C through a linear gradient of 1–3% bovine serum albumin in modified L-15 medium. After 2–3 h of sedimentation, 2.0-ml fractions were collected and examined using a haemocytometer. A 0.1-ml sample of a fraction rich in these axons was spread on a glass slide. Under Nomarski interference optics ($\times 256$), single axons were drawn into a glass micropipette with a tip of inner diameter 6–8 μm attached to a micromanipulator (c). About 100 axons were drawn into each micropipette and used for chemical analysis.

measured in the goldfish retina (1.8 ± 0.3 , D.M.K.L., unpublished).

If GABA is to be considered a transmitter candidate in cone horizontal cells, the specific activity of glutamate decarboxylase in them should be substantially higher than that in the whole retina. So, does the specific activity measured in isolated fusiform axons represent that in entire cone horizontal cells, which consist of both cell bodies and axons? Because isolated cone (external) and rod (intermediate) horizontal cell bodies could not be distinguished unequivocally, enzymatic activities in the cell bodies could not be measured directly. Since in cone horizontal cells of the cyprinid retina, however, most of the structures characteristic of the chemical synapse have been observed on the cell bodies and not on the fusiform axons^{5,14,15}, the isolated axons used in this study probably contained very few synaptic endings (Fig. 2). As glutamate decarboxylase has been shown to be very concentrated in presynaptic endings of neurones which presumably use GABA as a transmitter^{16,17}, the specific activity of glutamate decarboxylase in a cone horizontal cell complete with all its synaptic endings is probably much higher than that in an isolated

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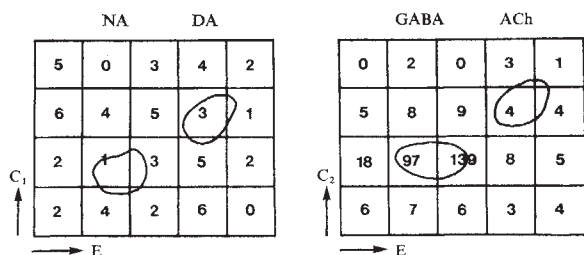


Fig. 3 Two-dimensional analyses of radioactive transmitters synthesised by 100 fusiform axons incubated with purified methyl-³H-choline chloride (6.7 Ci mmol^{-1}), and L-³H-glutamic acid (generally labelled, 2.5 Ci mmol^{-1}) and L-3, 5-³H-tyrosine (25 Ci mmol^{-1}) for 8 h at 24°C . After incubation, cells were lysed by drawing $1.0 \mu\text{l}$ of 1 M HCl containing unlabelled precursors and transmitters into the micropipette. Radioactive products were first separated by paper electrophoresis. Regions corresponding to noradrenaline (NA) and dopamine (DA) were analysed further by ascending paper chromatography using a solvent system C_1 : methylethyl ketone-propionic acid-water ($200:65:55$)¹¹. Regions corresponding to GABA and ACh were similarly analysed using another solvent system C_2 : *n*-butanol-acetic acid-water ($60:15:25$)^{11,12}. Each chromatogram was dried, stained and cut into 1-cm squares. Each square was placed in a scintillation vial containing $1 \text{ ml } 0.01 \text{ M HCl}$ for 1 h 9 ml of Aquasol was then added to each vial and the radioactivity was measured by scintillation counting¹⁸ (25% efficiency). The values represent net c.p.m. above a background of about 19 c.p.m.

fusiform axon. It is therefore reasonable to assume, on chemical and morphological grounds (Fig. 2), that the specific activity of glutamate decarboxylase in intact cone horizontal cells of the goldfish retina is substantially higher than that in the whole retina.

Lam and Steinman have shown that in the goldfish retina, a large amount of ³H-GABA is accumulated selectively by cone horizontal cell bodies and fusiform axons (Cajal's external and internal horizontal cells)¹⁸. In addition, both the accumulation of ³H-GABA into these cells and the endogenous level of GABA in the retina were influenced by light stimulation of the retina^{16,23}. This study complements those results and shows that besides having a mechanism to accumulate exogenous GABA selectively, cone horizontal cells also possess the machinery to synthesis GABA. These findings point to GABA as a transmitter candidate used by cone horizontal cells of the goldfish retina.

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β -Adrenergic stimulation of cyclic AMP and protein kinase activity in the thyroid

THE demonstration that the sympathetic nervous system can have a direct effect on the thyroid¹, and the increasing clinical use of adrenergic blocking agents in thyrotoxicosis have reawakened interest in the effects of catecholamines on the thyroid. We have therefore compared the effects of thyroid stimulating hormone (TSH) and isoproterenol, a β -adrenergic stimulator, on the activation of protein kinase within the thyroid by cyclic AMP. We have also compared the inhibitory effects of the β -blocker propranolol, and the α -blocker phentolamine in this system. We found that isoproterenol is stimulatory at concentrations as low as 10^{-8} M , and that *l*-propranolol, but not *d*-propranolol blocks this effect.

Slices of calf thyroid were incubated and then frozen as before². Intracellular cyclic AMP was determined by the method of Steiner *et al.*³, and expressed per mg of protein in the homogenate. Intracellular protein kinase activity in the frozen powdered tissue was determined as before² using a $2 \text{ mM EDTA}/5 \text{ mM KH}_2\text{PO}_4$ homogenising buffer, $\text{pH } 7.0$, to which $0.5 \text{ mM } 1\text{-methyl-3-isobutyl xanthine}$ and 200 mM NaCl had been added. The ratio of the activity without added cyclic AMP to the activity found when $1 \mu\text{M}$ cyclic AMP is added during the assay reflects the degree of intra-

Table 1 Effect of TSH and isoproterenol on cyclic AMP and protein kinase activity

	Cyclic AMP (pmol mg^{-1})	Activity ratio
Control	5.6 ± 0.5	0.33 ± 0.04
<i>dl</i> -Isoproterenol ($5 \times 10^{-5} \text{ M}$)	$16 \pm 1.0^*$	$0.63 \pm 0.03^\ddagger$
TSH (50 mU ml^{-1})	$65 \pm 8^\dagger$	$0.99 \pm 0.04§$
Isoproterenol + TSH	82 ± 7	1.03 ± 0.05

Means $\pm$ s.e.m., $n = 4$. The values for TSH alone are not significantly different from those when isoproterenol was added to TSH.

* $P < 0.005$ compared with control.

$^\dagger P < 0.01$ compared with control and < 0.01 compared with isoproterenol alone.

$^\ddagger P < 0.025$ compared with control.

$§ P < 0.001$ compared with control and < 0.01 compared with isoproterenol alone.

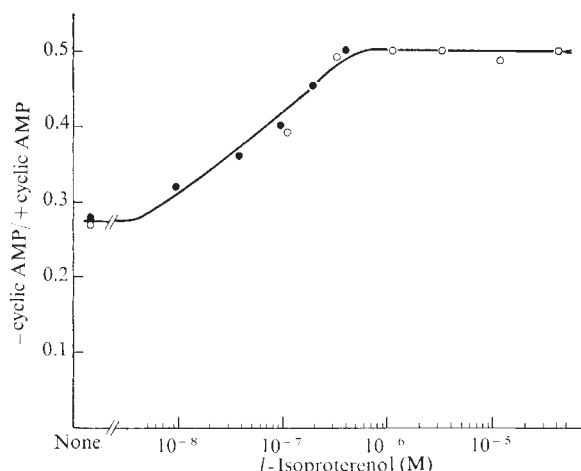


Fig. 1 Dose-response. Slices were incubated with various concentrations of *l*-isoproterenol for 10 min. A 20,000g supernatant from tissue homogenate was assayed for its ability to incorporate ^{32}P from ATP into mixed calf thymus histone. The 'activity ratio', the ratio of activity without added cyclic AMP to activity with $1\ \mu\text{M}$ cyclic AMP added to the assay reflects the degree of intracellular activation of the enzyme. $\circ$, Results from one experiment, performed in duplicate; $\bullet$, results from a second experiment, also performed in duplicate.

cellular activation of protein kinase. We refer to this measure of endogenous activation as the 'activity ratio'.

Thyroid slices were incubated with *l*-isoproterenol for various times. The activity ratio increased linearly from zero time to 2 min, then remained constant for at least 10 min. Subsequent incubations were carried out for 10 min, which was the time previously found for TSH at $50\ \text{mU ml}^{-1}$ to produce its maximal effect in this system². Figure 1 shows the response of the kinase activity ratio to various concentrations of *l*-isoproterenol. Concentrations as low as $10^{-8}\ \text{M}$ produced a significant increase in the activity ratio, and half maximal stimulation occurred at approximately $7 \times 10^{-8}\ \text{M}$.

Table 1 shows the relative effects of large doses of *dl*-isoproterenol ($5 \times 10^{-5}\ \text{M}$) and TSH ($50\ \text{mU ml}^{-1}$) on cyclic AMP and kinase activity (Table 1). TSH fully activated the kinase activity ratio (0.99), while *isoproterenol*, which only raised the cyclic AMP level to $16\ \text{pmol mg}^{-1}$, increased the kinase activity ratio to 0.63. The cyclic AMP level produced when *isoproterenol* and TSH were combined was not significantly greater than that produced by TSH alone.

To characterise the receptors involved, thyroid slices were preincubated with the β -adrenergic receptor blocking agent propranolol for 15 min. Control slices contained $6.8 \pm 0.6\ \text{pmol mg}^{-1}$ cyclic AMP and this level was unaltered by preincubation with $10^{-4}\ \text{M}$ *dl*-propranolol. TSH at $16\ \text{mU ml}^{-1}$, a submaximally stimulatory dose, raised the cyclic AMP concentration to $29 \pm 5\ \text{pmol mg}^{-1}$, which was also unaffected by propranolol. *Isoproterenol* ($5 \times 10^{-5}\ \text{M}$) raised

the cyclic AMP concentration to $16.8 \pm 1.4\ \text{pmol mg}^{-1}$, while propranolol reduced this response to 12.3 ± 1.0 ($P < 0.025$). The kinase activity ratio in the control slices in this experiment was 0.42 ± 0.01 and was unaltered by propranolol. *dl*-Isoproterenol ($5 \times 10^{-5}\ \text{M}$) raised the activity ratio to 0.64 ± 0.03 and this was reduced by propranolol to 0.50 ± 0.03 ($P < 0.005$). Preincubation of the slices with $10^{-4}\ \text{M}$ phentolamine, an α -adrenergic blocking agent, had no effect on the stimulation produced either by TSH at mU ml^{-1} (0.81 ± 0.04) or $5 \times 10^{-5}\ \text{M}$ *isoproterenol*.

Low concentrations of the purified isomers of propranolol were also compared for inhibitory effects on the stimulation produced by *l*-isoproterenol (Table 2). While $6 \times 10^{-6}\ \text{M}$ *l*-propranolol significantly lowered the cyclic AMP and kinase activity ratio, preincubation with *d*-propranolol actually enhanced the response to *isoproterenol*. The kinase activity ratio and the intracellular cyclic AMP concentrations correlated closely (Fig. 3).

In 1968, Gilman and Rall suggested that β rather than α -adrenergic receptors mediated the effects of adrenaline in bovine thyroid slices⁴. Subsequent studies on adrenergic stimulation of the thyroid have, however, been confusing

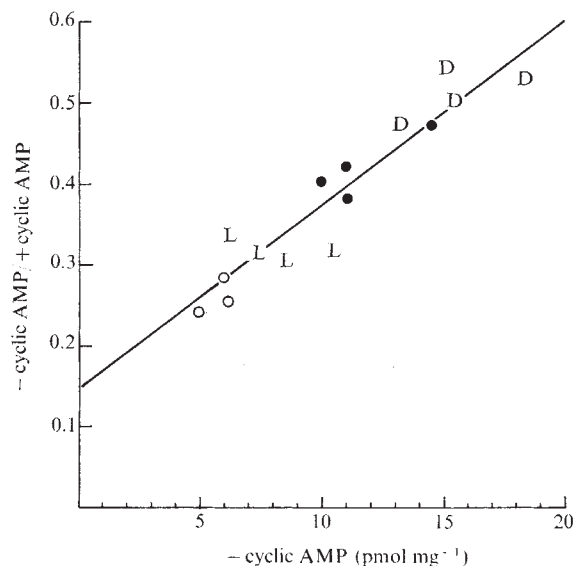


Fig. 2 Correlation of kinase activity and intracellular cyclic AMP. Slices were preincubated with *d*- or *l*-propranolol ($6 \times 10^{-6}\ \text{M}$) for 15 min then incubated with $6 \times 10^{-7}\ \text{M}$ *l*-isoproterenol for 10 min. The two isomers of propranolol had no significant effects in the absence of the *isoproterenol*. Correlation coefficient $r = 0.93$, activity ratio $= 0.023$ (cyclic AMP) ± 0.146 . $\circ$, Control slices; $\bullet$, slices incubated with *isoproterenol*, D indicates preincubation with *d*-propranolol and L indicates *l*-propranolol.

and apparently contradictory, but have been generally interpreted as indicating the adrenergic effects on the thyroid were mediated by α receptors⁵⁻⁸. The adrenergic blocking agents phentolamine and propranolol at concentrations greater than $10^{-4}\ \text{M}$ have even been reported to inhibit the effects of TSH on adenylyl cyclase in thyroid slices⁹⁻¹¹. These findings have been interpreted as a reflection of a direct pharmacological effect on membranes by these blocking agents¹¹. In this study, however, only *l*-propranolol was inhibitory, while both *d*- and *l*-propranolol exert membrane effects. The apparently synergistic effect of *d*-propranolol, while unexpected, is not unprecedented, as stimulatory effects of *d*-propranolol have been observed previously¹².

We have found previously that the activation of cyclic AMP-dependent protein kinase correlates well with increases in the level of intracellular cyclic AMP produced by TSH and prostaglandin E_1 in calf thyroid slices^{3,13}. Although the magnitude of the response of the activity

Table 2 Effect of *d*- and *l*-propranolol on the *isoproterenol*-mediated stimulation of cyclic AMP and protein kinase activity

	Cyclic AMP	Activity ratio
Control	6.0 ± 0.4	0.28 ± 0.02
<i>l</i> -Isoproterenol ($6 \times 10^{-7}\ \text{M}$)	$11.7 \pm 1.0^*$	$0.42 \pm 0.02^*$
<i>Isoproterenol</i> + <i>d</i> -propranolol ($6 \times 10^{-6}\ \text{M}$)	$15.9 \pm 1.0^\dagger$	$0.51 \pm 0.02^\dagger$
<i>Isoproterenol</i> + <i>l</i> -propranolol ($6 \times 10^{-6}\ \text{M}$)	$8.1 \pm 1.0^\ddagger$	$0.32 \pm 0.01^\ddagger$

* $P < 0.005$ compared with control.

† $P < 0.05$ compared with *isoproterenol* alone.

‡ $P < 0.005$ compared with *isoproterenol* alone.

Isoproterenol + *l*-propranolol was not significantly different from control.

ratio to β -adrenergic stimulation is clearly less than that produced by TSH, it is apparently faster, reaching its maximum effect within 2 min, as opposed to about 10 min for TSH². Adrenaline was reported to produce a half maximal response in cyclic AMP at a concentration of 3×10^{-7} M in calf thyroid slices¹, although iodide uptake could be stimulated by as little as 5×10^{-7} adrenaline in isolated bovine thyroid cells³. Our observation of an increase in kinase activity with as little as 10^{-8} M isoproterenol suggests that lower concentrations of this pure β stimulator are required than are needed for an effect of the combined α and β stimulator, adrenaline.

Comparison of the action of isoproterenol and TSH reveals that isoproterenol acts more rapidly but does not raise cyclic AMP or stimulate kinase activity to the same degree as TSH. These findings, as well as the lack of effect of propranolol on TSH suggest that the two agents act on different receptor sites. The inhibition of the kinase response to isoproterenol by *l*- but not *d*-propranolol, as well as the lack of effect of phentolamine, indicates that the isoproterenol effect is mediated by β receptors.

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Influence of the pineal on wound healing

REMOVAL of the pineal gland affects endocrine functions in both birds and mammals, and has been shown to cause increased growth and spread of malignant tumours in the rodent. There are several hypotheses as to the mechanism by which the pineal affects these processes¹⁻⁴, but no role for the gland or its hormonal product melatonin has been suggested in normal processes. We have now found that pinealectomy can slow wound healing and that this effect can be reversed by treatment with melatonin.

Twenty 4-week-old inbred C57BL mice were divided into four groups of five. The mice in group 1 were pinealectomised; group 2 was sham-operated; group 3 received melatonin (1 mg kg⁻¹ body weight) intraperitoneally daily, beginning 24 h after pinealectomy and continuing for 10 d; and group 4 served as untreated controls. All animals were kept in transparent plastic cages in a 10 × 12 foot room at 74-78 °F (23-26 °C) with 40% relative humidity. A 60-W bulb provided light during the day, and was turned off at night. The animals were fed Rockland hamster diet and water *ad libitum*.

Pinealectomy and sham operation were performed as described previously², and 10 d later a 2-mm semicircular wound was made in the right ear of each animal, including the controls. After 72 h, colchicine (2 mg kg⁻¹ body weight) was injected intraperitoneally. All animals were killed 48 h later, always at 1030, to control for diurnal variations in pineal hormone

Table 1 Original data transformed to square root

	Control	Sham	Melatonin	Pineal
Observations (n)	250.00	250.00	250.00	250.00
Mean	2.09	2.15	2.25	1.58
Median	2.00	2.24	2.27	1.73
Variance	0.60	0.46	0.57	0.53

interaction. Daily weighing during the experiment showed no weight loss in any of the animals.

For the sake of uniformity, each wound was divided into three areas. Samples from the margins of each area were excised, fixed in neutral formalin, dehydrated and embedded in paraffin. All samples were treated simultaneously to reduce the chance of artefact to a minimum. Serial sections (7 μ m) were cut, then stained with haematoxylin and eosin. Each ear yielded 200 sections and all were mounted individually. We selected 50 slides from each area of each wound randomly, and counted the cells in each high-power field ($\times 40$); the average number in each field was constant from animal to animal. Obvious spindles and hyperchromatic nuclei with brush borders were considered to represent nuclei in mitosis and were included in the count.

We took as response (*y*) the number of mitoses per high-power field in each slide. Hence, the response was a discrete variable $0 \leq y \leq 20$ with 50 slides from each of five animals. We had 250 mitotic values for each group. The data showed that the responses did not follow normal distribution and the variances were heterogeneous. Therefore, the square root transformation was used in the final analysis. Table 1 summarises the transformed data. The ANOVA (Table 2) clearly indicates that at

Table 2 ANOVA using square root transformation

Sources of variation	Degrees of freedom	Sum of square	Mean square	F
Between groups	3	68.1248	22.7083	42.3
Between animals within groups	16	11.4246	0.7140	1.33
Residual	980	526.0679	0.5368	
Total	999	605.6173		

least two group means are significantly different at the 1% level of significance. To distinguish the pinealectomised groups from the others, a multiple comparison test was done. The result indicated that control, sham-operated, and melatonin-treated animals form one group, with means not significantly different, whereas the pinealectomised group differed significantly from the others at the 5% level.

The small number of mitoses in the healing wounds of pinealectomised mice is possibly the result of a direct hormonal effect on wound healing. This observation is in contrast to the previous reports of gonadal hypertrophy and increased growth of malignant tumours after the removal of the pineal²⁻⁴. The gonadal hypertrophy has been postulated to be the result of hyperplasia or of hypertrophy of individual cells of the target organ. In the case of transplantable malignant tumours, hormonal influence has been proposed as one of the causes of increased growth and spread.

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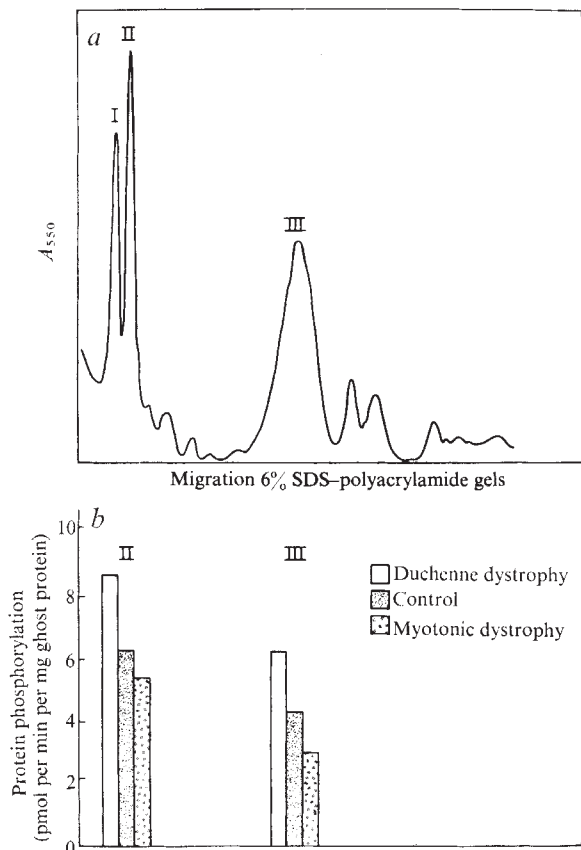
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Membrane protein kinase alteration in Duchenne muscular dystrophy

DUCHENNE muscular dystrophy (DuD) is the severe form of heredo-familial muscular dystrophy inherited as a sex linked recessive trait. The primary clinical manifestations are progressive muscle weakness usually starting in the pelvic girdle accompanied by hypertrophy in the calf musculature. Significant cardiac abnormalities and mental retardation have also been reported. The primary inherited metabolic defect is unknown¹. Elevated levels of muscle enzyme activities in the serum and their depletion in the muscle tissue have, however, long pointed to an abnormality in the plasma membrane as the probable site of the genetic defect.

To explore potential membrane alterations in DuD we used a strategy which had been successful in another common form of hereditary familial myopathy, myotonic muscular dystrophy (MyD). Changes of denervation, atrophy, fatty infiltration, or fibrosis may make muscle membrane alterations impossible to interpret unless correlated with similar changes in other tissues. So we used red cell membranes to explore the membrane abnormality in studies of MyD. MyD red cell ghost phospholipids, polypeptides, and carbohydrates were similar to age and sex matched controls. Endogenous protein phosphorylation, however, was decreased in fresh as well as frozen red blood cell membranes derived from patients with MyD^{2,3}.

Fig. 1 *a*, 6% SDS-polyacrylamide gel scan of solubilised erythrocyte membrane protein. No differences were demonstrated in scanning patterns from control, MyD, DuD or DuD carriers. Molecular weights: peak I, 210,000; II, 200,000; III, 90,000–100,000. *b*, Mean phosphorylation of major protein bands (II and III) of erythrocyte membrane measured under assay conditions (see text). Statistical data reported in Table 1.



Other investigators have similarly demonstrated that clinically inapparent red cell alterations may accompany muscle disorders. Layzer and coworkers have noted that the red cell contains a phosphofructokinase (PFK) composed of a muscle M-type subunit as well as a red blood cell or R-type subunit. In the inborn error of metabolism affecting this enzyme the muscle M subunit is absent in red cell as well as the muscle and may be used as an index of the genetic defect⁴. Morphological studies have similarly suggested red blood cell involvement in muscular dystrophy. Using scanning electron microscopy, Matheson and Howland reported specific changes in the shape of RBCs derived from patients with DuD⁵. In our own studies, we find no specificity. Similar alterations were noted in red blood cells from patients with MyD, myotonic congenita, oculopharyngeal muscular dystrophy, homozygotes for DuD as well as carriers of DuD (S. E. Miller, A.D.R., and S.H.A., submitted for publication). Although our studies do not show that the morphological alterations are specific, they indicate clearly that the red blood cell membrane is involved in many muscular disorders.

In this study, our initial intent was to determine whether the alterations in membrane protein kinase noted in MyD were specific for that disorder or were nonspecific alterations as noted in our morphological studies. With the subsequent demonstration of a different level and pattern of protein phosphorylation in DuD, we were able to use this sensitive enzymatic reaction to monitor the membrane abnormality.

Heparinised blood was obtained from DuD and MyD patients and matched with control heparinised blood. Seven DuD patients from five families, three DuD carriers from three families, and seven MyD patients from four families were used. Erythrocyte ghosts were prepared as previously described^{2,6}. All assays were performed on fresh ghosts without freezing or storage. Incubations were performed as previously described and all assays were run in duplicate on 6% SDS-polyacrylamide gels. Phosphorylation of the major erythrocyte protein peaks (II and III) (Fig. 1) was linear for the incubation time of 1 min, and the protein concentration used. [γ -³²P]-ATP was not limiting under these conditions^{2,3}.

There were no reproducible differences in the protein polyacrylamide gel patterns of erythrocyte ghosts from DuD or DuD carriers from either MyD or controls. Similarly phospholipid, fatty acid, and Ca²⁺-ATPase activities were not significantly different².

The phosphorylation of protein bands II and III in DuD membranes was significantly increased compared with controls or MyD patients (Fig. 1 and Table 1). No differences were noted between young male controls matched with DuD and the mixed adult control group matched with MyD and DuD carriers. These data have been pooled in Table 1. Our previous demonstration of decreased phosphorylation of band III in MyD was confirmed in this series of experiments³. Also no difference was again demonstrated in the phosphorylation of MyD band II compared with controls.

DuD carrier band II phosphorylation was 8.58 ± 1.03 pmol per mg ghost protein per min. This value is increased significantly compared with controls but is the same value as noted in DuD patients. The similarity of the phosphorylation of DuD and DuD carrier band II phosphorylation suggests that one gene dose may be sufficient to reveal alterations in the endogenous protein kinase activity but may not be sufficient to produce clinical dystrophy. Mild elevation of serum creatine phosphokinase activity in many carriers have been interpreted as reflecting partial leakage of the muscle membrane⁷. Endogenous protein kinase activity may be closely related to the primary defect in DuD and represent a highly sensitive index of membrane alteration. But further kinetic studies as well as specific substrate and enzymatic isolations will be necessary before definitive genetic interpretations may be made.

These results demonstrate that abnormalities of protein

Table 1 Phosphorylation of erythrocyte protein bands

	II pmol mg ⁻¹ min ⁻¹	<i>P</i>	III pmol mg ⁻¹ min ⁻¹	<i>P</i>
Duchenne (<i>n</i> = 7)	8.62 ± 0.72	< 0.005	6.25 ± 0.68	< 0.01
Control (<i>n</i> = 16)	6.29 ± 0.36		4.31 ± 0.32	
Myotonic (<i>n</i> = 7)	5.40 ± 0.48	NS	3.00 ± 0.48	< 0.05

Transfer of ³²P from [γ-³²P]-ATP to erythrocyte membrane protein band under assay conditions. *n*, Number of patients or controls. *P* is determined by Student's *t* test.

phosphorylation are present in red cell membranes from patients with DuD and DuD carriers as well as from patients with MyD, and the specific pattern of alterations is distinctive for each disorder. Our studies of MyD demonstrated that muscle membranes possessed a similar decrease in protein kinase activity as had been noted in red blood cell membranes⁸. Our present results combined with previous morphological and biochemical data suggest that DuD like MyD is an inherited disorder of membranes with widespread tissue involvement.

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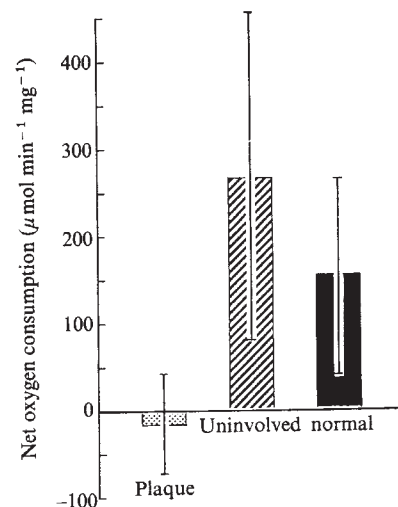


Fig. 1 Oxygen consumption of skin homogenates. Aliquots of skin homogenate obtained from normal control patients and from plaque and uninvolved skin of psoriatic patients were treated as described in the text. Oxygen consumption in the presence of dioxigenase and arachidonic acid alone was subtracted from the oxygen consumption in the presence of added homogenate and the difference is recorded as $\mu\text{mol O}_2 \text{ min}^{-1} \text{ mg}^{-1}$. The difference in oxygen consumption between psoriatic plaque and uninvolved psoriatic skin is significant ($P < 0.001$) (Student's *t* test for small sample populations) whereas no statistically significant difference was noted between oxygen consumption in psoriatic uninvolved skin and normal control skin ($P > 0.1$).

present in this blanched area (our unpublished results). These observations suggested that a diffusible inhibitor of PG synthesis was present within the psoriatic lesion. We now report the detection of an inhibitor(s) of PG synthesis in psoriatic plaque that is not present in extracts of uninvolved skin obtained from either psoriatic patients or normal volunteers.

Punch biopsies (4 mm across) were obtained from psoriatic plaques (eleven) and uninvolved skin (nine) of psoriatic patients and from normal volunteers (eight) after injection of 1% xylocaine. Skin specimens were homogenised in 100 mM Tris-HCl buffer, pH 8.5. Oxidation of arachidonic acid by sheep vesicular gland dioxigenase was carried out as described by Smith and Lands^{4,5}. Dioxigenase was prepared by homogenising aliquots of acetone powder of sheep vesicular gland (Upjohn) in 100 mM Tris-HCl buffer, pH 8.5. Enzyme preparations and skin homogenate were placed in the side of the electrode holder of an oxygen monitor. Immediately thereafter, arachidonic acid (Sigma, 20 μM final concentration) was added to the mixture and the reaction rate ($\mu\text{mol O}_2 \text{ min}^{-1}$) was determined by continuous measurement of oxygen uptake, using an oxygen electrode at 37 °C. Protein was determined by the method of Lowry *et al.*⁶ using bovine serum albumin as the standard.

In our assay system, normal human skin homogenates contained substances that increased oxygen consumption (Fig. 1). The nature of this stimulation in oxygenation of arachidonic acid is unknown but is under study. Oxygen consumption of psoriatic plaque homogenates was markedly depressed compared with that of normal appearing skin obtained from psoriatic patients or normal control patients (Fig. 1). Oxygen consumption in the presence of psoriatic plaque extracts was less than that of dioxigenase and substrate alone (control), suggesting a direct interaction between an inhibitor(s) in the psoriatic plaque and dioxigenase; decreased oxygen consumption may also reflect an absence of nonspecific alternate reactions that consume oxygen. Clinical observations coupled with other data (our unpublished results) indicate that the area surrounding the psoriatic lesion is deficient in PGE₂ during ultraviolet light therapy. Our results suggest that the reduction of oxygen consumption in the presence of homogenate from

Inhibitor(s) of prostaglandin synthesis in psoriatic plaque

In 1926, Woronoff¹ described an area of blanched skin surrounding psoriatic lesions. The mechanisms of this reaction were unknown but were thought to be a consequence of vasoconstriction. In considering the probable explanation of the blanched area, we have noted that the blanched area is approximately the same width (1 cm) around the psoriatic lesion irrespective of the size of the lesion (suggesting that a substance diffuses out of the lesion), and the whitened area does not become red during ultraviolet light therapy. Snyder and Eaglstein reported that the redness following exposure to ultraviolet light can be aborted by treatment with indomethacin, which is known to inhibit the synthesis of prostaglandins (PGs^{2,3}). We have also found that: (1) PGE₂ levels in whole skin removed from the blanched area are one-third of those in normal skin; (2) injection of PGE₂ into the blanched area produced redness, and (3) an inhibitor(s) of PG synthesis was

psoriatic lesions is the result of inhibition of sheep vesicular dioxigenase.

The detection of an inhibitor(s) of PG synthesis explains the clinical phenomenon of the blanched area surrounding psoriatic plaques. The relationship of this inhibition to the psoriatic process is unknown. Studies are under way to define further the nature of the inhibitor(s), and preliminary experiments show that it is dialysable, not susceptible to ribonuclease or deoxyribonuclease, and not destroyed by proteases.

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Role of macrophages in *in vitro* induction of T-helper cells

MACROPHAGES are of importance in the initiation of immune responses, both *in vitro*¹ and *in vivo*^{1,2}. Both immune responses involving B cells, such as antibody production³⁻⁶, and those involving T cells, such as the lymphocyte transformation⁷, the mixed lymphocyte reaction (MLR) (refs 8 and 9), and the induction of cytotoxic responses¹⁰ require macrophages. Various concepts of macrophage function in immune induction have been suggested. The simplest concept proposed is that macrophages augment lymphocyte survival *in vitro*¹¹. Although this is one of their functions *in vitro*, it does not explain fully their role *in vitro* and especially *in vivo*. In the induction of antibody responses, two functions were defined, the breakdown of antigens to the appropriate size⁵ and the presentation of antigen to B cells in an optimally immunogenic manner¹². Because the T-cell reactions already studied, such as the MLR, involve ill-defined antigens, the surfaces of living cells, even less is known about the mechanisms of macrophage function in the stimulation of T cells than of B cells.

Having recently described a system which enables unprimed T cells to be activated by soluble protein antigens and become helper cells¹³, we investigated whether macrophages are involved in this reaction and attempt a definition of their role. We report that macrophages were necessary, that there are genetic restrictions for effective T-macrophage interaction and that macrophages can be replaced by the supernatants of antigen-incubated macrophages of the same or an F₁ strain. The genetic restrictions for effective T-macrophage interaction, however, did not apply if a particulate form of the antigen, keyhole limpet haemocyanin (KLH), conjugated to Sepharose beads (S-KLH), was used, indicating the existence of at least two different cellular pathways resulting in the generation of helper cells.

Purified T cells were obtained from cortisone-resistant thymus cells (CRT) of CBA mice by the nylon wool technique¹⁴, which removes adherent cells (macrophages and B cells). To generate helper cells, 15×10^6 adherent cell-depleted CRT were incubated with KLH for 4 d, together with syngeneic (CBA), allogeneic (BALB/c) or semi-allogeneic F₁ (CBA $\times$ BALB/c) macrophages obtained from peritoneal exudate. In a second stage (cooperation), 10^5 of these cells were incubated together with 15×10^6 normal spleen cells and trinitrophenylated KLH (TNP-KLH). After 4 d, the anti-dinitrophenyl (DNP) or anti-TNP response was measured by counting plaque-forming

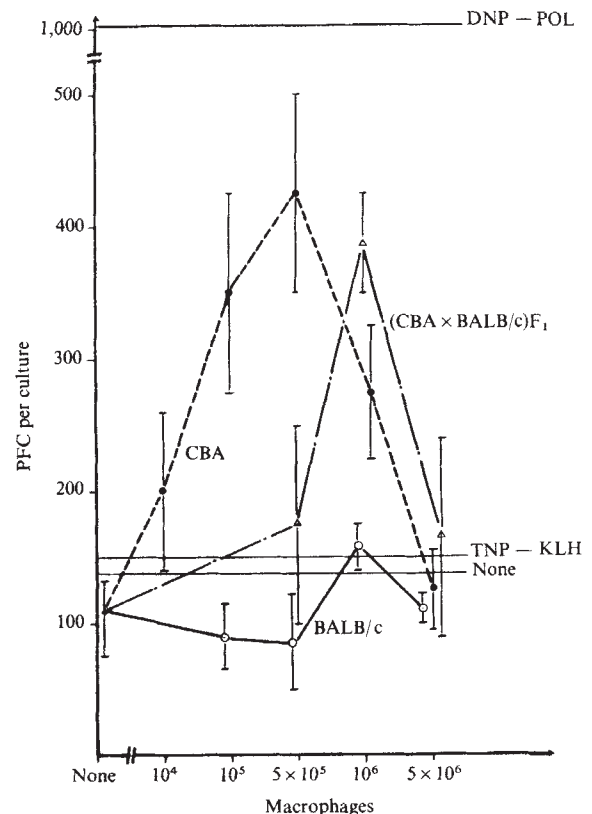


Fig. 1 Requirement for syngeneic or semi-allogeneic macrophages in the induction of T-helper cells. PE from CBA, BALB/c and (CBA $\times$ BALB/c)F₁ mice were used as a source of macrophages. To stimulate PE, mice had been injected with 1 ml 10% sterile Proteose-Peptide broth (Oxoid Code L46) 3 or 4 d previously. Unstimulated PE were just as effective as stimulated PE. The PE were washed in saline and treated with a sheep anti-T serum (dilution 1 : 4) and spleen cell adsorbed guinea pig complement (1 : 8) for 45 min at 37 °C. After washing, macrophages were incubated in graded numbers with 15×10^6 nylon wool purified CRT cells from CBA mice and 0.1 μ g KLH in 1 ml HEPES-buffered Eagle's MEM containing 10% foetal calf serum in small (1 cm) Marbrook inserts for 4 d at 37 °C in an atmosphere of 10% CO₂ in air. The outside compartment contained 25 ml of bicarbonate-buffered MEM with 5% normal calf serum. As a control, no macrophages were added to the purified CRT. After 4 d incubation the cells were collected, washed and 10^5 viable cells added to 15×10^6 CBA spleen cells containing 0.1 μ g ml⁻¹ TNP-KLH in Marbrook flasks (as above) and incubated for 4 more d. The effect of macrophages on the generation of helper cells was measured by the cooperation between the carrier-activated T cells and the hapten-activated B cells expressed by the IgM plaque-forming ability of B cells against DNP or TNP-coated SRBC. In our cultures, unprimed B cells do not make IgG.

cells (PFC) against TNP- or DNP-coated sheep red blood cells (DNP-SRBC) thereby giving a measure of the helper activity generated in the first culture.

Figure 1 shows that no helper cells were generated in the absence of macrophages, as indicated by the number of PFC which fell in the background range. With the addition of increasing numbers of syngeneic macrophages to adherent cell-depleted CRT the helper activity increased as shown by the number of PFC generated. Addition of 5×10^5 macrophages gave the highest number of PFC, indicating optimal helper activity. A response was not seen when macrophages from BALB/c mice were incubated with CBA T cells, but was seen when they were incubated with BALB/c T cells (P. E., and M. F., unpublished), indicating that either T cells and macrophages must be histocompatible or that allogeneic macrophages exert an inhibitory effect on the helper cell induction. The latter possibility has been excluded, since helper cells are obtained, if a mixture of CBA and BALB/c macrophages are incubated with

Table 1 Capacity of supernatant from syngeneic or semi-allogeneic macrophages (incubated with KLH for 4 d) to induce helper cells

First culture			Second culture		
Helper cell induction			Cooperation		
T cells (Macrophage-depleted spleen)	Antigen	Macrophage or supernatant	Helper cells transferred 10 ⁵	Challenge	Anti-DNP-response (PFC $\pm$ s.e.)*
+	KLH	Nil	+	TNP-KLH	65 $\pm$ 35
+	KLH	5 $\times$ 10 ⁵ macrophage	+	TNP-KLH	274 $\pm$ 39†
+	Nil	CBA S/N ₄ KLH(5%)	+	TNP-KLH	284 $\pm$ 34§
+	KLH	CBA S/N ₄ (40%)	+	TNP-KLH	70 $\pm$ 5
+	KLH	CBA S/N ₄ (20%)	+	TNP-KLH	93 $\pm$ 52
+	KLH	CBA S/N ₄ (1%)	+	TNP-KLH	62 $\pm$ 17
+	Nil	BALB/c S/N ₄ KLH(5%)	+	TNP-KLH	68 $\pm$ 28
+	Nil	F ₁ S/N ₄ KLH(10%)	+	TNP-KLH	293 $\pm$ 48
+	Nil	F ₁ S/N ₄ KLH(1%)	+	TNP-KLH	167 $\pm$ 40
—	—	—	—	TNP-KLH	123 $\pm$ 38†
—	—	—	—	DNP-POL	647 $\pm$ 142
—	—	—	—	Nil	111 $\pm$ 41

Spleen cells were used for the induction of T-helper cells. The adherent and phagocytic cells were removed by incubating 200 $\times$ 10⁶ spleen cells (in 10 ml of HEPES-buffered MEM containing 10% foetal calf serum) with 0.1 g carbonyl-iron in a 25 ml glass beaker for 1 h at 37 °C, by keeping a strong magnet on the bottom of the beaker while pouring the cells carefully into another beaker. The magnet treatment was repeated once, the cells washed and cultured in small (1 cm) Marbrook vessels with or without antigen (KLH, 0.1 μ g ml⁻¹) and macrophage or macrophage supernatant. To obtain pure macrophage supernatant, 5 $\times$ 10⁶ CBA, BALB/c or F₁ PE were incubated in the wells of a Linbro culture plate (No. FB-16-24 TC, Biocult, Glasgow) for 2 h at 37 °C, the non-adherent cells removed by extensive washings with HEPES-buffered MEM. Residual T and B cells were removed by treating with undiluted anti-T and anti-B serum and complement for 45 min at 37 °C and washing twice with MEM. The remaining cells (macrophage) were 99.7% phagocytic as judged by uptake of polystyrene beads. The macrophages were then incubated with 25 μ g KLH (S/N₄ KLH) or no KLH (S/N₄) in 1 ml of bicarbonate-buffered MEM containing 10% foetal calf serum for 4 d at 37 °C. The supernatant (S/N) was then removed, spun for 15 min at 20,000g and frozen until use (1 ml supernatant was taken as 100%). Before use the supernatant was filtered through 0.45 μ m Millipore filter. For the cooperative response, 10⁵ viable cells from the first culture were incubated with 15 $\times$ 10⁶ normal spleen cells containing 0.1 μ g ml⁻¹ TNP-KLH in Marbrook flasks and incubated for 4 d at 37 °C. Dinitrophenylated polymeric flagellin (1.0 μ g ml⁻¹) was used as a control. The anti-DNP response was measured by the IgM plaque-forming ability of the B cells against DNP-coated SRBC.

* Antibody-forming cells per culture $\pm$ s.e. Results are pooled from three experiments.

† Used as control for calculation of statistics by Student's *t* test. Levels of significance: ‡ *P* < 0.05; § *P* < 0.01; || *P* < 0.02.

CBA T cells (P. E., and M. F., unpublished). F₁ macrophages were able to generate helper cells, although more semi-allogeneic macrophages were necessary to give a response. But this response was always less than with syngeneic macrophages. The need for syngeneic or semi-allogeneic cells for a successful macrophage-lymphocyte interaction was previously described in guineapigs¹⁵, but was not found in mice^{16,17}.

In a second set of experiments it was ascertained that macrophages incubated in the presence of antigen, for example, KLH, can release factor(s) able to induce specific helper cells to KLH. Table 1 shows that supernatant taken from purified macrophages, which had been incubated with KLH for 4 d, adequately replaced macrophages and antigen when tested in the helper system. Thus in this system no direct contact between macrophages and T lymphocyte is necessary. Moreover, in accordance with the findings described above, the supernatant raised from allogeneic (BALB/c) macrophages were ineffective,

whereas that derived from F₁ macrophages activated helper cells; and that obtained from syngeneic macrophages incubated in the presence of KLH had no effect, even in high doses.

By contrast, the latter supernatant was effective if used with a particulate antigen, KLH bound to agarose (Table 2), suggesting the existence of different mechanisms of helper cell activation by soluble and particulate antigens. Moreover, the supernatant from allogeneic macrophages had the capacity to induce helper cells, if tested in the presence of KLH bound to agarose (P. E., and M. F., unpublished). The chemical nature and biological significance of a 'specific' factor(s) which replaces antigen and macrophages in helper cell induction, and a nonspecific factor(s) which replaces macrophages, but only in the presence of particulate antigens, remains to be investigated. There are several reports of such nonspecific replacing factors¹⁸⁻²¹, but factors derived from macrophages, capable of replacing both macrophages and antigen have not been

Table 2 Capacity of supernatant from macrophages (incubated for 4 d with KLH bound to sepharose) to induce helper cells

First culture			Second culture		
Helper cell induction			Cooperation		
T cells (macrophage-depleted spleen)	Antigen*	Macrophage or supernatant	Helper cells transferred (10 ⁵)	Challenge	Anti-DNP-response (PFC $\pm$ s.e.)
+	S-KLH	Nil	+	TNP-KLH	83 $\pm$ 27†
+	S-KLH	5 $\times$ 10 ⁵ macrophage	+	TNP-KLH	277 $\pm$ 68‡
+	S-KLH	S/N ₄ KLH(5%)	+	TNP-KLH	265 $\pm$ 34‡
+	S-KLH	S/N ₄ (5%)	+	TNP-KLH	285 $\pm$ 43‡
—	—	—	—	TNP-KLH	77 $\pm$ 19
—	—	—	—	DNP-POL	634 $\pm$ 161
—	—	—	—	Nil	73 $\pm$ 23

* KLH was bound to Sepharose 2B using cyanogen bromide. Sepharose beads (10⁴ per culture) were used. TNP-KLH was used at 0.1 μ g ml⁻¹, DNP-POL at 1.0 μ g ml⁻¹. Results are pooled from three experiments.

† Used as control for calculation of statistics by Student's *t* test. ‡ Level of significance: *P* < 0.02.

described previously. The analysis of the genetic requirements for T lymphocyte-macrophage interaction should also be of interest since other forms of cell interaction have recently been suggested to have genetic (H-2-lined) restrictions, for example, T-B cooperation²² and the killing of virus-infected cells²³.

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Centromeric asymmetry and induction of translocations and sister chromatid exchanges in mouse chromosomes

THE mechanisms involved in chromosome rejoining are not understood. We showed that chromosome rejoining in Robertsonian centric fusion can be studied cytologically¹ with a staining technique based on the quenching by 5-bromodeoxyuridine (BrdU) of the fluorescence of the dye 33258 Hoechst². When mouse cells were grown for one generation in the presence of BrdU and stained with 33258 Hoechst, there was a brightly fluorescent spot appearing over half of the centromeric region in every autosome³. (This asymmetry presumably reflects the unequal distribution of thymidine (45% compared with 22%) between the two chains of mouse satellite DNA⁴, the fluorescent spot representing the old thymidine-rich chain of satellite DNA.) The arrangement of fluorescent spots in metacentric chromosomes resulting from Robertsonian fusion suggested that the thymidine-rich chain of satellite DNA in the centromeric region is associated with the same DNA chain (in terms of polarity) in every mouse autosome¹. We have used the centromeric asymmetry in mouse chromosomes as a marker for DNA polarity in studies on radiation and drug-induced chromosomal translocation and sister chromatid exchange (SCE).

Mouse cells from two permanent lines (RAG and A9) were grown in Eagle's medium with 10% foetal calf serum. For radiation studies, cells growing in Falcon tissue culture flasks were exposed to X rays (350 rad, 190 kV). Thirty minutes before irradiation colcemid (0.45 µg ml⁻¹) was added to the cultures, and 3 h after irradiation BrdU (10⁻⁵M)

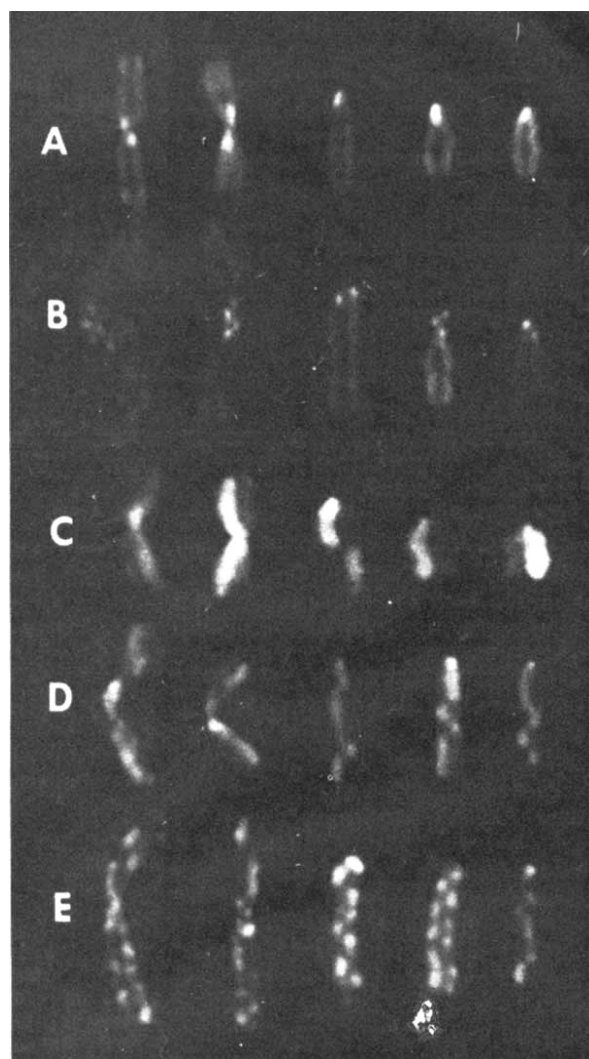


Fig. 1 Fluorescent staining of X-ray-induced dicentric chromosomes. RAG cells were grown for one cycle of replication in BrdU before fixation and staining with 33258 Hoechst. *a* and *b*, telocentric and metacentric chromosomes from unirradiated cells. *c* and *d*, Telo-dicentric and telo-meta-dicentric chromosomes from irradiated cells, showing the fluorescent spots on the opposite sides of the chromosomes. *e* and *f*, The same as (*c*) and (*d*), but with the fluorescent spots on the same sides of the chromosomes. *g*, Chromosomes from irradiated cells, showing SCE within the centromeric region.

or a mixture of BrdU (10⁻⁵M), fluorodeoxyuridine (2 × 10⁻⁵M) and uridine (3 × 10⁻⁴M) was added. The cells were fixed 24-25 h after irradiation. For studies with drugs, cells were grown in medium containing mitomycin C (MC) (0.5 µg ml⁻¹) and BrdU (10⁻⁵M) for either 22-24 h or 48 h and then fixed. In one experiment, cells were grown for 18 h in BrdU alone and then for 22-24 h in BrdU and MC together. The techniques for chromosome preparation and analysis have been described previously^{2,3}.

The relationship between DNA polarity and chromosomal rejoining in X-ray induced translocations was examined cytologically, using the fluorometrically detectable centromeric asymmetry (Fig. 1*a* and *b*) as a cytological marker for the chain of DNA with a given polarity. In non-irradiated RAG cells, end-to-end translocations were not seen. But, one generation after irradiation, (distal) end to (distal) end translocations appeared in more than 25% of metaphases. Both telo-dicentric and telo-meta-dicentric chromosomes (resulting respectively from translocation between two telocentric or between telocentric and metacentric chromosomes) were observed (Fig. 1*c-f*). (Isodicentric chromosomes

resulting from iso-chromatid breakage and proximal union should not be present until the second metaphase after irradiation⁵.) In every telocentric unit, a brightly fluorescent spot was associated with half of the centromeric region. In every metacentric unit two bright centromeric spots were arranged contralaterally. In the telo-dicentric chromosomes, the bright spots in the two centromeric regions could be arranged *a priori* either on the same side (relative to the long axis) or on opposite sides of the chromosome. In more than 95% of the telo-dicentric chromosomes, however, the bright spots were located on opposite sides of the chromosomes (Table 1). Similar results were obtained with the telo-meta-dicentric chromosomes. Since the spots presumably were associated with the DNA strand of the same polarity in every autosome, these results suggest that the orientation of the joined chromosomes in end to end translocations is such that DNA polarity is maintained from one centromere through the point of joining to the other centromere. The small proportion of telo-dicentric chromosomes in which the two bright spots were located on the same side of the chromosome (fewer than 5%) could be the result of X-ray-induced SCE at some point between the centromeres.

In the analysis of non-irradiated A9 cells, a stable chromosome aberration (appearing in more than 90% of the cells) was observed. The novel chromosome appears to have arisen from end-to-centromere translocations involving three chromosomes. After one cycle of replication in BrdU, the chromosome had three brightly fluorescent spots, all on the same side (Fig. 2). Assuming that this chromo-



Fig. 2 Fluorescent staining of a spontaneous chromosomal aberration. A9 cells were grown for one cycle of replication in BrdU before fixation and staining with 33258 Hoechst. Each chromosome is from a different cell.

some arose by translocation, these results suggest that the three chromosomes involved in the translocation were oriented such that the polarity of the DNA was maintained through the three regions of satellite DNA. (The stability of this translocation suggests that two of the three centromeres in this chromosome are either non-functional or absent.)

Our results suggest that DNA polarity is involved in determining the orientation of chromosomes during end-to-end and end-to-centromere translocations. This is consistent with the results of previous studies on other types of chromosomal reorganisation^{1,5,6}.

We have also used centromeric asymmetry to study SCE. The effects of X rays and MC, both previously shown to increase the frequency of SCE⁷⁻⁹, were investigated. In RAG cells grown for one generation in BrdU, SCE within the centromeric region (appearing as a split in the fluorescent spot) occurred with a very low frequency (0.05 per metaphase). At the first division after X irradiation (Fig. 1g) the frequency of SCE within the centromeric region increased to 0.3 per metaphase. After one cycle of replication in MC plus BrdU (Fig. 3b), the frequency increased to 11.2 per metaphase.

In contrast to the centromeric region, SCE in the non-centromeric region was not observed at all after one cycle of replication in MC plus BrdU (Fig. 3b). After two cycles of replication in MC plus BrdU, however, the effect of MC

Table 1 Arrangement of fluorescent spots in the centromeric region of X-ray-induced dicentric chromosomes

Arrangement of spots	Telo-dicentric	Telo-meta-dicentric*	Total
Opposite side	730	92	822
Same side	32	10	42

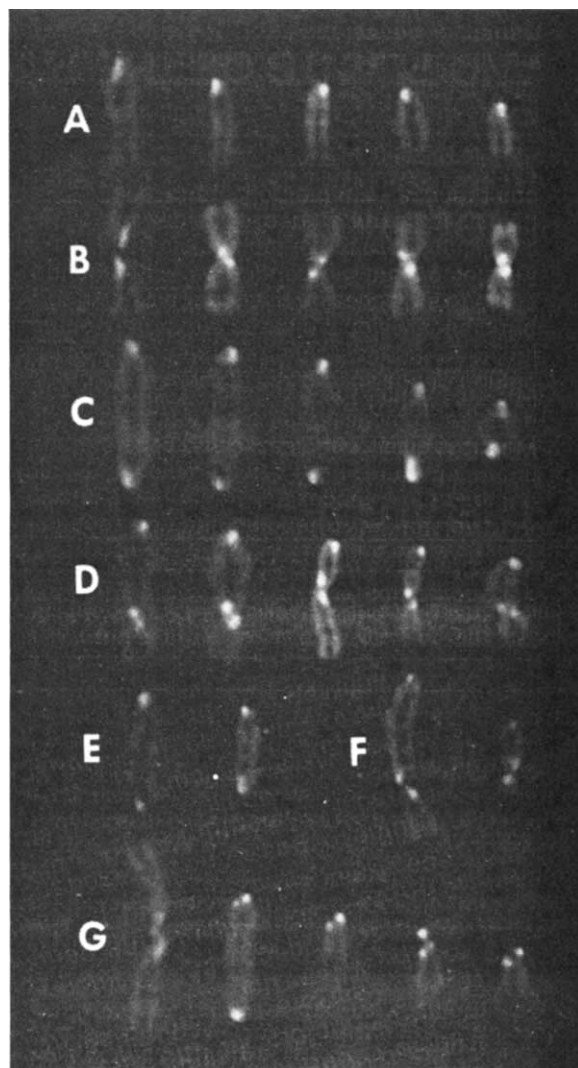
RAG cells were irradiated and grown for one generation in BrdU and stained as described in the text. The data were collected from 292 metaphases, all of which contained at least one dicentric chromosome.

* For these chromosomes, the arrangement refers only to the spots associated with the two centromeric regions on either side of the point of joining.

was obvious (Fig. 3e). RAG cells grown for two cycles in BrdU without MC had on the average 12 SCE per metaphase; RAG cells grown for two cycles in BrdU with MC had more than 200 SCE per metaphase.

The difference between the appearance of MC-induced SCE in the centromeric and non-centromeric regions is not caused by a difference in the effect of MC in the two regions. If cells were grown for one generation in BrdU and then for a second generation in MC plus BrdU, there was a marked increase in the frequency of SCE (to 92 per

Fig. 3 Fluorescent staining of MC induced SCE. RAG cells were grown for different times in MC plus BrdU before fixation and staining with 33258 Hoechst. a, Cells grown for one generation in BrdU without MC. b, Cells grown for one generation in MC plus BrdU, showing SCE only in the centromeric region. c, Cells grown for two generations in BrdU without MC. d, Cells grown for two generations in BrdU, with MC present only during the second generation. e, Cells grown for two generations in MC plus BrdU.



metaphase) in the non-centromeric as well as the centromeric region (Fig. 3d). Thus MC-induced SCE in the non-centromeric region can be observed in the first division following MC treatment, but only if BrdU is added one generation before MC. The failure to observe MC-induced SCE in the non-centromeric region until the second metaphase when MC and BrdU are added together is in agreement with autoradiographic results that suggested that SCE involves exchanges of double stranded DNA molecules rather than exchanges of single polynucleotide strands¹⁰. The ability to detect SCE in the centromeric region at the first metaphase when MC and BrdU are added together is due presumably to the asymmetric distribution of thymidine in the two strands of mouse satellite DNA. This natural asymmetry thus provides a system in which SCE can be detected within the first generation after labelling, which could facilitate certain types of studies on chromosome breakage and rejoining.

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Non-repetitive DNA transcripts in nuclei and polysomes of polyoma-transformed and non-transformed mouse cells

WE have determined the extent to which non-repetitive DNA is represented in nuclear and polysomal RNA of polyoma-transformed and non-transformed mouse cells in culture. Our RNA-DNA saturation-hybridisation experiments have shown that in transformed cells, polysomes contain only 10-20% of the informational complexity present in nuclear RNA. Similar results were obtained with RNA from confluent cultures of non-transformed cells. In subconfluent cultures of non-transformed cells, however, polysomes contained about 40% of the sequence diversity present in nuclear RNA. Further experiments, with appropriate RNA mixtures, suggest that there are also qualitative differences in polysomal RNA between transformed and non-transformed cells, as well as between confluent and subconfluent cultures of non-transformed cells.

The results of experiments using RNA from the nuclei of PY AL/N cells and from polysomes prepared by centrifugation through discontinuous sucrose gradients are shown in Fig. 1. On the assumption that only one strand of DNA is transcribed the saturation values were taken as twice the intercept values of the double-reciprocal plots. The value obtained for nuclear RNA (30%) is the same as that reported earlier using total RNA from these cells⁴. The average saturation value for polysomal RNA was 6.4% (four experiments, range 5.2-8.0%).

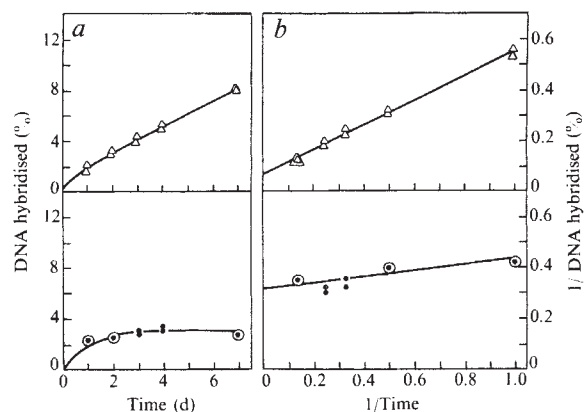


Fig. 1 *a*, Time course of hybridisation reactions using RNA from nuclei (Δ) and polysomes ($\bullet$) of PY AL/N cells. Data are corrected for DNA self-reassociation as determined from controls which contained DNA alone⁴. *b*, Double-reciprocal plots of the data in (*a*). The procedures for isolating and characterising non-repetitive DNA labelled with thymidine-2-¹⁴C have been published⁴. To obtain RNA, cells were removed from the bottles with 0.05% trypsin+0.02% EDTA, washed twice in serum-free medium and resuspended at 8×10^6 cells ml⁻¹ at 0°C in 10 mM Tris, pH 8.4, containing 0.14 M NaCl and 1.5 mM MgCl₂. Nuclear and cytoplasmic fractions were prepared using 0.05% Triton X-100². Nuclear RNA was obtained by hot phenol extraction¹. Polysomes were purified from the supernatant by centrifugation through discontinuous sucrose gradients⁷. Polysomal RNA was isolated by phenol-chloroform extraction⁸. Nuclear and polysomal RNA preparation were treated with DNase and passed over columns of Sephadex G-100 (ref. 1). Hybridisation reactions containing the appropriate RNA (12 mg ml⁻¹) and labelled non-repetitive DNA (12 μ g ml⁻¹) were carried out as previously described⁴.

Two experiments were carried out to determine the degree to which the polysomal RNA might be contaminated with RNA released from nuclei that were lysed or damaged during cell fractionation. First, DNA was labelled with thymidine-2-¹⁴C, and nuclei and polysomes were isolated as usual. Approximately 99.5% of the acid-insoluble counts remained in the nuclei; only 0.5% was found in the polysomes. The second experiment was based on reports^{2,3}, that when cells are labelled with uridine, no counts appear for 10-15 min in either polysomal messenger RNA or cytoplasmic ribosomal RNA. In cells labelled with uridine-2-¹⁴C for 10 min and fractionated as usual, the distribution of radioactivity between nuclei and polysomes was identical to that observed when DNA was labelled. When these nuclei were treated with DNase, lysed with SLS and centrifuged as in the preparation of polysomes, 14% of the acid-insoluble counts was recovered in the pellet. If this is a reasonable estimate of the maximum amount of nuclear RNA that could contaminate the polysomes, the 0.5% found above suggests an upper limit of contamination of about 3-4%.

A second set of experiments was carried out using more stringent criteria to define polysomal RNA^{4,5}. Polysomes were centrifuged through linear sucrose gradients and the material sedimenting at $>100S$ was collected. After treatment with EDTA, this fraction was again centrifuged through a sucrose gradient and the portion sedimenting at $<80S$ was collected and the RNA was extracted. The results of hybridisation experiments using such RNA are presented in Fig. 2. The saturation values for two experiments (3 and 4%) are in fairly good agreement with those obtained by Bishop *et al.*⁶ using the poly(A)-containing RNA from the cytoplasm of HeLa cells.

In the case of non-transformed cells it was necessary to collect from many bottles to obtain adequate quantities of RNA. Therefore, polysomes were prepared only by the procedure involving centrifugation through discontinuous sucrose gradients. In the experiments with transformed cells, polysomal RNA isolated by this method gave the highest

Table 1 Saturation values of RNA–non-repetitive DNA hybridisation experiments with RNA from non-transformed AL/N cells

Growth state	Nuclear RNA Average	(Range)	No. of experiments	Polysomal RNA Average	(Range)	No. of experiments
Confluent	20	—	1	5.7	(5.0–6.4)	3
Subconfluent	19	—	1	8.6	(8.4–8.8)	3

Each experiment used the appropriate RNA (12 mg ml^{-1}) and non-repetitive DNA ($12 \text{ } \mu\text{g ml}^{-1}$). Hybridisation reactions were carried out as described in Fig. 1. Assuming that only one strand of DNA is transcribed, the intercepts of double-reciprocal plots were doubled to obtain the tabulated values.

saturation values; consequently the measurements with non-transformed cell RNA are likely to be maximum estimates of the RNA sequence diversity in polysomes.

The saturation values obtained with nuclear and polysomal RNA of nontransformed cells are summarised in Table 1. As with transformed cells, the saturation values obtained with nuclear RNA from confluent and subconfluent cells agree with those previously obtained using total RNA from these growth states¹. The values for polysomal RNA from confluent (5.7%) and subconfluent (8.6%) cells suggest that more non-repetitive DNA is represented in the latter. Experiments using a mixture of RNA from the two growth states gave saturation values of 10.4% and 11.2%, implying qualitative as well as quantitative differences with respect to the sequence diversity of polysomal RNA in confluent and subconfluent cells.

Qualitative differences between the polysomal RNA species in transformed cells and subconfluent non-transformed cells are also suggested, since experiments with RNA mixtures yielded saturation values of 9.2% and 11.6%.

To determine whether the hybrids contained only non-repetitive DNA, the DNA was isolated from the hybrids and its reassociation behaviour was examined in the presence of an excess of unlabelled, unfractionated mouse DNA. As Figure 3 shows, except for 5% which bound to hydroxylapatite immediately after denaturation, the DNA from the hybrids reassociated as expected for nonrepetitive sequences. The labelled DNA used in these experiments contained some sequences resulting from DNA self-reassociation, as well as sequences from RNA–DNA hybrids. Even assuming that only 50% of the hybridised DNA originated from RNA–DNA duplexes

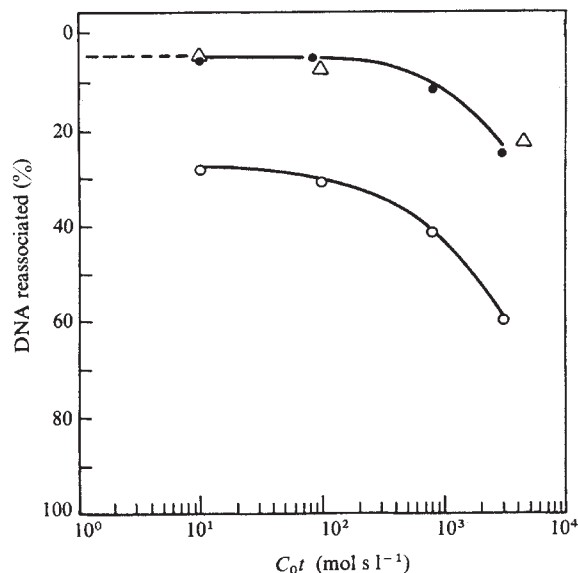


Fig. 3 Reassociation of DNA isolated from hybrids in two experiments. Hybridised DNA was isolated from hydroxylapatite and the RNA was removed by alkaline hydrolysis. The DNA was dialysed into distilled water. Sheared, unlabelled and unfractionated mouse embryo DNA was added to the sample to a concentration of 1.0 mg ml^{-1} and the ionic strength was adjusted to 0.12 M phosphate buffer. The sample was denatured and incubated at 60°C . Samples were removed periodically and assayed on hydroxylapatite. The reassociation of the unlabelled DNA was determined by the A_{260} (○), and that of the labelled DNA on the basis of its radioactivity (● and △).

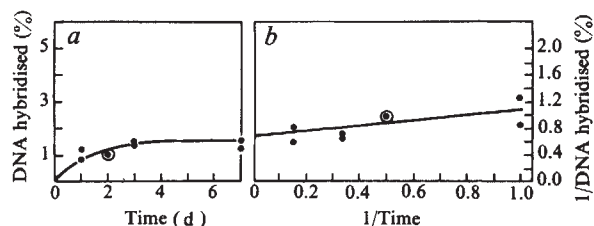


Fig. 2 *a*, Time course of hybridisation experiments using the $>100\text{S}$ PY AL/N RNA that sediments at $<80\text{S}$ after EDTA treatment. *b*, Double-reciprocal plot of the data in (*a*). Hybridisation conditions were similar to those in Fig. 1. Cells were lysed, nuclei removed, and polysomes isolated as described in Fig. 1. Polysomes suspended in buffer (10 mM Tris, $\text{pH } 7.4$, containing 10 mM NaCl and 8 mM MgCl_2) supplemented with polyvinyl sulphate ($50 \text{ } \mu\text{g ml}^{-1}$), hereafter referred to as B+PVS, were layered over 30 ml , linear $15\text{--}30\%$ (w/v) sucrose gradients (sucrose in B+PVS) built over a 2.0 ml cushion of 60% sucrose. After centrifugation in a SW27 rotor at $25,000 \text{ r.p.m.}$ and 4°C for 3 h , the material sedimenting at $>100\text{S}$ was pooled and diluted $1:2$ with B+PVS, and the polysomes were pelleted by centrifugation overnight in a type 60 Ti rotor at $55,000 \text{ r.p.m.}$ and 4°C . Polysomes were resuspended in B+PVS and the solution was made 25 mM EDTA. The treated polysomes were layered over $15\text{--}30\%$ linear gradients and centrifuged as before. The material sedimenting at $<80\text{S}$ was collected, MgCl_2 was added to 5 mM , and the polysomes were precipitated by adding ethanol to 50% and storing overnight at -10°C . The polysomes were collected by centrifugation at $25,000\text{g}$ for 15 min and resuspended in 10 mM Tris, $\text{pH } 7.4$, containing 0.1 M NaCl, 1 mM EDTA and 0.5% SLS. RNA was isolated by phenol–chloroform extraction⁸.

and that all the material which initially bound to hydroxylapatite occurred in this fraction, the results still indicate that at least 90% of the DNA which hybridised with RNA was comprised of non-repetitive sequences.

To assess the precision of base pairing in the hybrids formed, their thermal stability was determined. Samples were bound to columns of hydroxylapatite and eluted as a function of temperature at 5°C intervals. The thermal dissociation patterns resembled those obtained for total RNA–DNA hybrids¹; the midpoints of the transitions were $79\text{--}80^\circ\text{C}$. We concluded that no extensive mismatching of base pairs occurred in these hybrids.

The results of these experiments can be summarised as follows. First, although the saturation values obtained in RNA–DNA hybridisation reactions show a dependence on the method used for polysome isolation, only a fraction of the non-repetitive DNA transcripts found in the nucleus of either transformed or non-transformed cells are also found in the polysomes. Second, confluent and subconfluent cultures of non-transformed cells are identical in the amount of non-repetitive DNA represented in nuclear RNA. On the other hand, both quantitative and qualitative differences exist in the non-repetitive transcripts in the polysomes of cells in these growth states. Third, the nuclear RNA of the virus-transformed cells contains a more diverse population of non-repetitive DNA transcripts than does the nucleus of the non-transformed cells. In contrast, non-repetitive DNA is represented equally in the polysomes of transformed and

non-transformed cells. Qualitative differences exist, however, in the polysomal sequences of these two types of cell.

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Different susceptibility of DNA and RNA to cleavage by metal ions

METAL ions are required in virtually every phase of genetic information transfer, and they are generally essential components of biochemical processes involving DNA and RNA. Under certain conditions, however, metal ions can have deleterious effects, and one of these, the degradation of polyribonucleotides, is not frequently considered by those who deal with nucleic acids in the presence of metals. No such degradation has been demonstrated with DNA, although it is known¹ that DNA, like RNA, is subject to thermal degradation, and therefore can be expected to undergo some degradation also in the presence of metal ions. It is important to understand the relative susceptibility of polydeoxynucleotides and polyribonucleotides to degradation by metal ions to answer the following questions. To what extent is DNA, compared with RNA, vulnerable to metal ion degradation during biochemical experiments? What are the implications for metal ion toxicology? Is the difference in susceptibility to metal hydrolysis sufficient to make possible a quantitative separation of degraded RNA from undegraded DNA in a DNA-RNA mixture? Finally, does this difference in susceptibility reflect differences in the intrinsic stabilities of DNA and RNA, and perhaps throw light on the reason for the evolutionary selection of DNA, rather than RNA, as the favoured bearer of the primary genetic information? These questions can be answered by a comparison of DNA and polyribonucleotide degradation by metal ions using a method of detection sensitive enough to respond to the first break in the macromolecule.

Polyribonucleotides are readily degraded by heating in the presence of various metal ions^{2,3}. Zinc(II) ion³ is a very effective degrading agent, and the characteristics and mechanism of the zinc reaction have been thoroughly investigated³⁻⁵. The zinc degradation of poly(rA) is typical of that of polyribonucleotides². For these reasons we have chosen to compare the degradation, in the presence and absence of zinc(II) ion, of poly(rA) with that of calf thymus DNA. The non-degradability of DNA by metal ions has been suspected^{3,6,7} on the basis of assays which could, however, detect only very extensive cleavage. By following the zinc cleavage reaction by sedimentation velocity measurements, in conditions of strand separation, we can detect even one break per strand. The reactions were carried out at pH 7.0, low ionic strength, with and without 2 Zn per nucleotide residue, and, for comparison of single and double stranded DNA, at each of two temperatures: 50 °C, which is below the melting range of the DNA under these conditions, and 80 °C, which is above it⁷. (Poly(rA) is single stranded at both temperatures^{8,9}.)

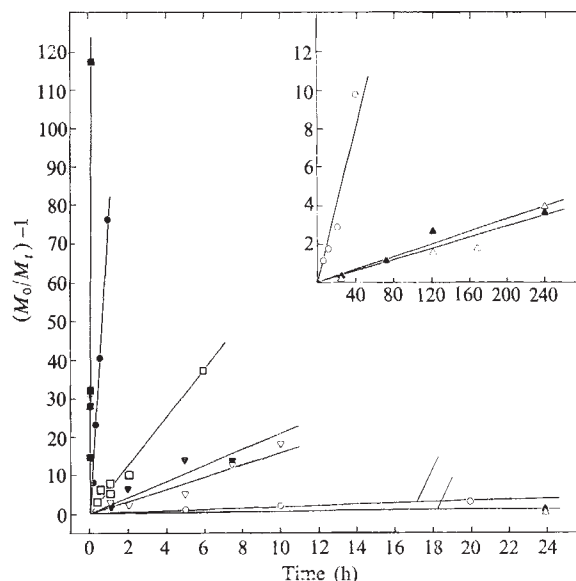


Fig. 1 Time course of cleavage of calf thymus DNA and poly(rA) at 50 °C and 80 °C, pH 7, with and without 2 Zn per nucleotide residue, as measured by reduction of the weight average, single-strand molecular weight during times t from the initial value M_0 to values M_t . DNA: 50 °C, ($\blacktriangle$) with Zn, ($\triangle$) without; 80 °C, ($\blacktriangledown$) with Zn, ($\triangledown$) without. Poly(rA): 50 °C, ($\bullet$) with Zn, ($\circ$) without; 80 °C, ($\blacksquare$) with Zn, ($\square$) without. Calf thymus DNA ($M_0 = 1.2 \times 10^6$) was from Worthington Biochemicals; the stock solution was prepared as before¹. Poly(rA) ($M_0 = 0.41 \times 10^6$) was from Miles Laboratories. Reaction mixtures contained 0.10 mM nucleotide residue and 5 mM NaNO_3 , and zinc ion was introduced as $\text{Zn}(\text{NO}_3)_2$; pH was initially adjusted to 7.0 by addition of NaOH. For sedimentation measurements, zinc was removed from DNA solutions by batch exchange on Dowex-50(Na), and then to separate the strands¹² NaOH was added to 0.1 M and NaCl to 1 M; poly(rA) solutions were made 0.01 M in sodium EDTA buffer, pH 7.0 and 0.1 M in NaCl⁸ (poly(rA) remains single stranded under such conditions^{8,9}). Weight average sedimentation coefficients were determined at 20 °C (Beckman Model E ultracentrifuge, ultraviolet absorption optics) from the movement of the half-maximal position of the integral boundary pattern. Weight average molecular weights were calculated: for DNA, using the correlation of Studier¹² for the alkaline medium, $S_{20,w} = 0.0528 (M^{0.40})$; for poly(rA), from the correlation of Fresco and Doty¹³ for neutral 0.15 M NaCl, $S_{20,w} = 0.024 (M^{0.45})$ (S in Svedbergs).

The sedimentation results, in terms of the calculated weight average, single-strand molecular weights, are presented in Fig. 1. It is immediately obvious from Fig. 1 that a large difference in susceptibility to zinc cleavage exists between single or double stranded DNA, and poly(rA), so that very extensive zinc depolymerisation of poly(rA) occurs during a time in which DNA sustains negligible damage. Some cleavage of the DNA—both with and without zinc—was eventually detected, even at the lower temperature (inset of Fig. 1), but the effect of zinc on DNA cleavage was insignificant, both at 50 and 80 °C, compared with its effect on the cleavage of poly(rA).

All the plots of Fig. 1 fit straight lines reasonably well. Table 1 provides quantitative comparisons of the slopes of these plots, which represent rates of depolymerisation on a single strand basis. The Table also lists times required for reduction of the molecular weight to given fractions of the initial value. Table 1 shows that there was no significant added effect of zinc on the cleavage of DNA, either above or below the melting temperature, while there was a 200-300-fold increase in the rate of depolymerisation in the case of poly(rA). The rate for poly(rA) was 4,000 times greater than the rate for DNA, in the presence of zinc, when the DNA was double stranded (at 50 °C). When the DNA was single stranded (at 80 °C), poly(rA) was still degraded 1,000 times more rapidly than DNA. In the absence of zinc the degradation of poly(rA) was only 14 times faster at 50 °C and four times faster at 80 °C.

These results indicate that cleavage induced directly by metal

Table 1 Rate of reduction of the weight average, single-strand molecular weight of calf thymus DNA, and of poly(rA), at 50 °C and 80 °C, pH 7.0, with and without 2 Zn per nucleotide residue

	Slope (h ⁻¹) of M_0/M_t against t^*		Approximate time (h) for reduction of $M_0^\dagger$			
	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn
DNA						
50 °C	0.016±0.002	0.014±0.003	62	71	560	640
80 °C	2.1±0.6	1.6±0.2	0.48	0.62	4.3	5.6
Poly(rA)						
50 °C	72±4	0.20±0.04	0.014	5.0	0.12	45
80 °C	1,400±400	6.2±0.6	0.00064	0.16	0.0062	1.4

* Evaluated from the plots of Fig. 1 (the mean deviation for fit to the experimental points are appended). M_0 , initial weight average molecular weight; M_t , values at times t .

† Calculated from the slope of M_0/M_t against t ; $M_0/M_t = 1 + (\text{slope})t$.

ions is generally unlikely during *in vitro* manipulations of DNA, although it must be taken into account with polyribonucleotides. Figure 1 and Table 1 can serve as a guide to the amount of damage to be expected under various conditions.

The great difference in the susceptibility of polyribonucleotides, compared with polydeoxynucleotides, to zinc cleavage makes possible the quantitative removal of RNA from a DNA-RNA mixture. This becomes evident when one calculates from the data of Table 1 that, at 50 °C with zinc, about 5,000 breaks occur in a poly(rA) strand for one break in a DNA strand. Selective zinc degradation of RNA has already been utilised¹⁰ in connection with chromatin reconstitution studies; our results indicate that the DNA components remain intact during such degradation.

Our results suggest also that DNA should be much less susceptible than RNA to deleterious action by metal ions *in vivo*. Thus the question arises whether these effects may be part of the reason for the predominant evolutionary selection of DNA, instead of RNA, as the bearer of the primary genetic information.

Obviously, any attempt to answer this question, as any question involving evolutionary hypotheses, must be speculative. It has been suggested¹ that the relatively small difference in thermal stability of DNA and RNA can account for the choice of the DNA duplex as the repository of genetic information. But this difference in thermal stability is only about one three-hundredth the difference in stability to metal ions. Moreover, metal ion degradation of polyribonucleotides proceeds⁵ through a mechanism that involves the 2' OH group, the presence of which in RNA and absence in DNA constitutes the only primary structural difference between the two types of nucleic acid. For both of these reasons, if stability of the primary genetic material is a criterion of its evolutionary selection, susceptibility towards metal ions seems much more important than susceptibility towards heat. It is of course possible that evolutionary selection of the structure was in response to pressures other than stability and coincidentally resulted in DNA with high stability to metal ion degradation.

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Amounts of isoaccepting lysine tRNAs change with the proliferative state of cells

VARIATIONS in isoaccepting tRNA populations occur in mammalian cells during development, neoplasia, and virus infection, and such changes may reflect the involvement of tRNA in regulatory processes^{1,2}, although firm evidence to that effect is not available. Ortwerth and Liu³ have shown that in normal or neoplastic cells an isoaccepting species of lysine tRNA, tRNA₄^{Lys}, is present in dividing cells but absent from non-dividing cells. Ortwerth *et al.*⁴ determined some of the functional properties of tRNA₄^{Lys} from various tissues and obtained evidence that the species is a real isoacceptor of tRNA^{Lys} rather than an artefact. Normal, proliferating cells that were examined had only relatively small amounts of tRNA₄^{Lys}; however, a larger proportion of the isoacceptor was found in neoplastic cells such as mouse leukaemia and Morris hepatoma cells. It is desirable, therefore, to establish whether the large amount of tRNA₄^{Lys} is peculiar to the neoplastic state or whether normal cells may also have large amounts of that isoacceptor under some growth conditions. Therefore, we studied isoaccepting lysyl-tRNA profiles from mouse cells in different states of proliferation: adult liver, embryo and growing and quiescent primary cultures of embryonic cells. We found that normal growing cells also have an appreciable amount of tRNA₄^{Lys}.

Ten-day-old mouse embryos, with heads and extremities removed, were prepared for monolayer tissue culture by dispersing cells with trypsin and seeding in roller bottles in Eagle's MEM⁵ containing penicillin G (100 U ml⁻¹), streptomycin (100 µg ml⁻¹) and 10% foetal calf serum. tRNA and aminoacyl-tRNA ligases were isolated from primary cells, embryo and liver as previously described⁶ and tRNA was purified further by DEAE-cellulose chromatography⁷. tRNA was aminoacylated with lysine as before⁸.

In mouse leukaemia cells in suspension culture, tRNA₄^{Lys} is 47% of the total tRNA^{Lys}, and this amount decreases to 16% as cell density increases and proliferation stops³. Comparable data on the amount of tRNA₄^{Lys} in a uniform population of rapidly dividing normal cells and the effect of increasing cell density were not available. We have compared the Lys-tRNA^{Lys} profiles of growing and density-inhibited quiescent primary cultures of embryonic cells. The results (Fig. 1a) indicate a much larger amount of tRNA₄^{Lys} in growing cells than in resting cells. Consistent with these results is the finding of an appreciable amount of tRNA₄^{Lys} in embryonic cells which were not in tissue culture (Fig. 1b). These results also indicate that placing the cells in tissue culture does not in itself lead to an altered tRNA^{Lys} profile. In contrast to results with proliferating cells, tRNA₄^{Lys} is present only in a very small amount in adult mouse liver (Fig. 1c).

The results are summarised in a quantitative manner in Table 1. tRNA₄^{Lys} varies from 3% in adult mouse liver to 24% in proliferating monolayer culture. Similar results with cultured murine sarcoma virus-transformed cells (H. J., D. J., and C. H., unpublished) and SV40-transformed cells (J. Katze, personal communication) have been obtained. In both cases, tRNA_{5a}^{Lys} (ref. 8) seems to be present in addition to tRNA₄^{Lys} in rapidly proliferating cells and decreases as the cell population becomes

Table 1 Effect of state of growth of cells on chromatographic distribution of isoacceptors of lysine tRNA

Cells	Growth state	Distribution of lysine tRNA isoacceptors			
		1	2	4	5
Adult mouse liver	Quiescent	—	58	3	38
Primary mouse embryo	Quiescent	2	50	9	40
Mouse embryo	Growing	4	37	15	44
Primary mouse embryo	Growing	2	35	24	38

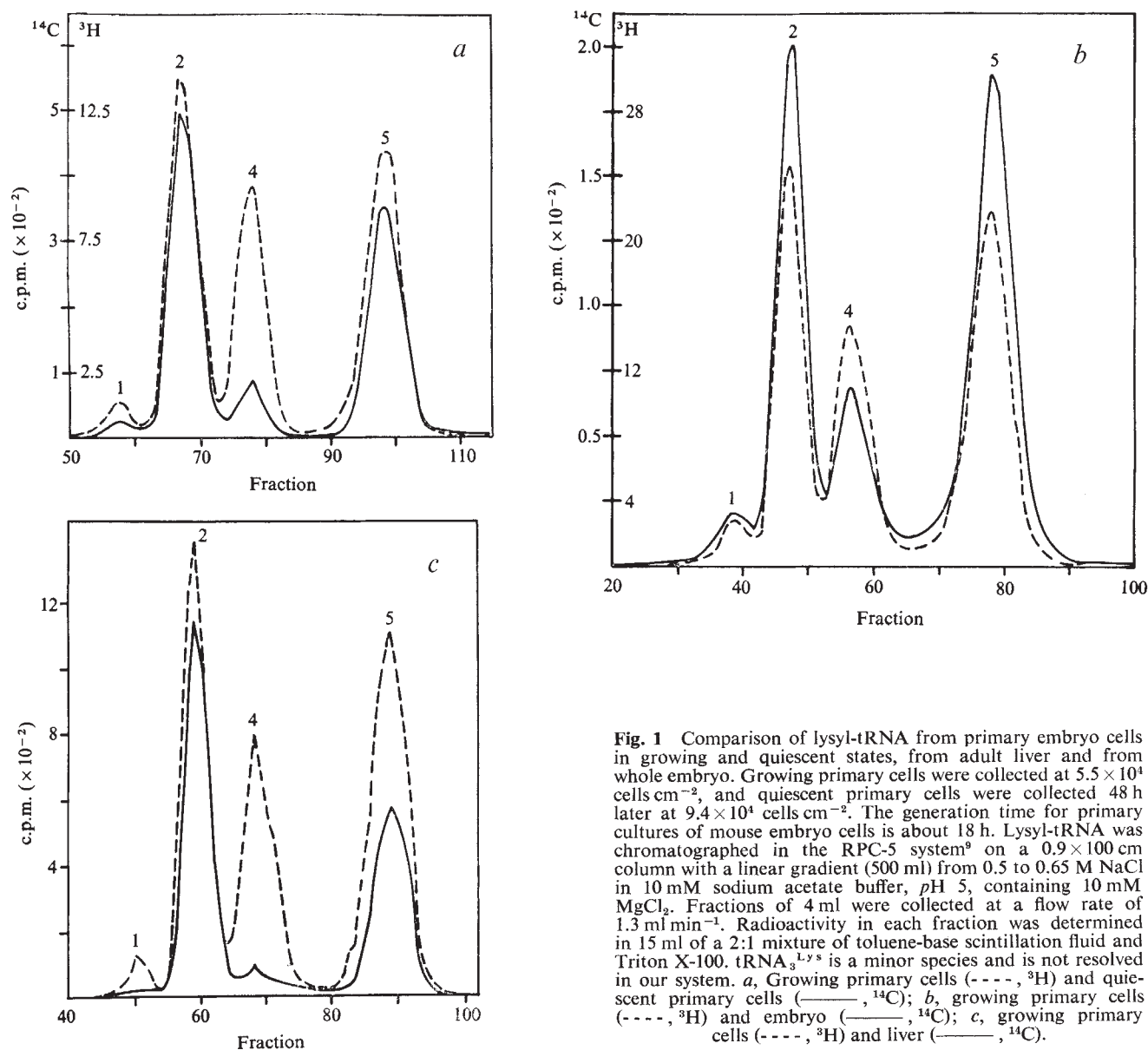


Fig. 1 Comparison of lysyl-tRNA from primary embryo cells in growing and quiescent states, from adult liver and from whole embryo. Growing primary cells were collected at 5.5×10^4 cells cm^{-2} , and quiescent primary cells were collected 48 h later at 9.4×10^4 cells cm^{-2} . The generation time for primary cultures of mouse embryo cells is about 18 h. Lysyl-tRNA was chromatographed in the RPC-5 system⁹ on a 0.9×100 cm column with a linear gradient (500 ml) from 0.5 to 0.65 M NaCl in 10 mM sodium acetate buffer, pH 5, containing 10 mM MgCl_2 . Fractions of 4 ml were collected at a flow rate of 1.3 ml min^{-1} . Radioactivity in each fraction was determined in 15 ml of a 2:1 mixture of toluene-base scintillation fluid and Triton X-100. $\text{tRNA}_3^{\text{Lys}}$ is a minor species and is not resolved in our system. *a*, Growing primary cells (---, ^3H) and quiescent primary cells (—, ^{14}C); *b*, growing primary cells (---, ^3H) and embryo (—, ^{14}C); *c*, growing primary cells (---, ^3H) and liver (—, ^{14}C).

dense. (The presence of $\text{tRNA}_{5\text{A}}^{\text{Lys}}$ is not always detected by RPC-5 chromatography, but benzoylated DEAE-cellulose chromatography provides resolution⁸.) The effect of cell density on profiles of other isoaccepting tRNAs has been noted¹⁰.

It is now clear that the distribution of isoaccepting species of tRNA^{Lys} in both normal and neoplastic cells varies with the proliferative state of cells. The constancy of the sum of percentages of $\text{tRNA}_2^{\text{Lys}}$ and $\text{tRNA}_4^{\text{Lys}}$ suggests that species 2 and 4 are structurally related with differences attributed to the degree of modification⁴. Confirmation of that possibility and investigations into functional differences are the subjects of continuing investigations.

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Errata

In the article "An ancient lunar magnetic dipole field" by S. K. Runcorn (*Nature*, **253**, 701; 1975) the legends to Fig. 2a and b were transposed.

In the article "A gene cluster in *Aspergillus nidulans* with an internally located *cis*-acting regulatory region" by H. N. Arst and D. W. MacDonald (*Nature*, **254**, 26; 1975) there was an error in Fig. 1. The two arrows linking L-proline and L- Δ^1 -pyrroline-5-carboxylate should be reversed—so that the top arrow points to the right and the bottom to the left.

matters arising

Cooling velocity and cell recovery

FARRANT *et al.*¹ have suggested that two separate processes determine the survival of cultured cells thawed from -196°C storage, namely a holding temperature of about -26°C preceding the subsequent storage temperature. We report that optimal recovery of cultured cells after freezing depends on an optimal cooling velocity^{2,3} rather than temporary attainment of a particular subzero protective temperature zone¹ before storage in liquid nitrogen at -196°C .

Normal human diploid fibroblasts were cultured in Dulbecco's modified Eagle's (DME) medium with 16.6% foetal calf serum, 20 mM HEPES buffer, penicillin, streptomycin and neomycin. Cells were suspended and dispensed into glass ampoules (cryules, Wheaton Glass Company) each containing 10^6 cells ml^{-1} of medium and either 5% or 10% DMSO as described previously³.

In pilot experiments, using an electric thermometer whose tip reached into an ampoule filled with 1 ml of the cryoprotective medium, we measured the time required to cool an ampoule from $+25^{\circ}\text{C}$ to -25°C . This was done either by immersion of the ampoule in liquid nitrogen followed by rapid transfer at -25°C to a methanol bath set at -26°C or immersion in the methanol bath directly. The time required was 30 s or 2.5 min, respectively.

We designed the freezing experiments as follows: (1) rapid cooling from $+25^{\circ}\text{C}$

to -25°C within 30 s followed by maintenance at -26°C for 1 h; (2) slow cooling from $+25^{\circ}\text{C}$ to -25°C within 2.5 min followed by maintenance at -26°C for 1 h; (3) slow cooling from $+25^{\circ}\text{C}$ to -25°C within 2.5 min followed by immediate immersion in liquid nitrogen. After 1 h, the ampoules of series (1) and (2) were also rapidly immersed in liquid nitrogen. All experiments were performed with two different concentrations of DMSO (5% and 10%).

Table 1 shows survival of only 0.75% of the cells cooled rapidly to -26°C (cooling velocity about $100^{\circ}\text{C min}^{-1}$) and maintained there for 1 h before immersion in liquid nitrogen; 52.7% survival when cooled more slowly to -26°C (cooling velocity of about $20^{\circ}\text{C min}^{-1}$) and then immediately immersed in liquid nitrogen; 61.2% survival after cooling slowly to -26°C and maintained at this temperature for 1 h before transfer to liquid nitrogen.

We have previously shown that optimal recovery of frozen human diploid fibroblasts from storage at -196°C is achieved if the cooling velocity at subzero temperatures down to -30°C is about 1.5 to $4.5^{\circ}\text{C min}^{-1}$ (ref. 3). Furthermore, cells do not cool at a constant rate in the range of $+10^{\circ}\text{C}$ and -30°C (Fig. 1).

The assumption that the freezing velocity is the most important parameter for recovery agrees with Farrant *et al.*¹, except for one experimental detail. They state that (starting at room temperature) -26°C had been reached in less than 1 min, while optimal recovery of frozen

cells required at least 5 min immersion in a bath at -26°C . This is inconsistent with our results (experiment 1) and does not demonstrate conclusively the existence of a protective temperature zone. As our results show, on the one hand, nearly optimal recovery can be reached without keeping cells at a protective temperature zone (experiment 3) and on the other almost no viable cells remain even though they were kept at a recommended protective temperature for 1 h if the initial freezing rate was too high.

Farrant *et al.*¹ happened to apply the optimal cooling rate for their cells when they immersed their glass test tubes for some time at -25.6°C , whereas -22°C ,

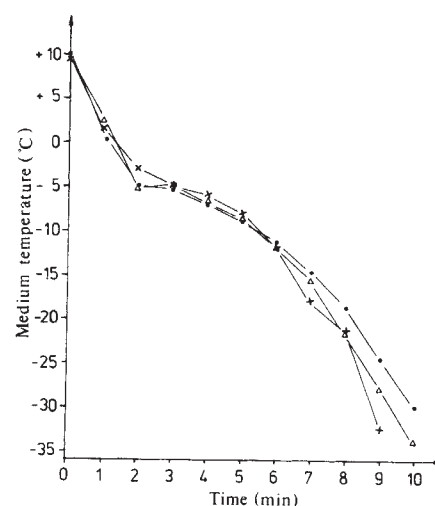


Fig. 1 Cooling rates of three independent experiments using ampoules containing 1 ml of tissue culture medium.

Table 1 Survival rate of human diploid fibroblasts following different freezing procedures

DMSO in medium (%)	Time for cooling $+25^{\circ}\text{C}$ to -26°C	Time held at -26°C	Number of cells recovered		Recovery rate (%)
			Viable	Non-viable	
5	30 s	1 h	$5,084 \pm 1,987$	$752,230 \pm 78,352$	0.67
10	30 s	1 h	$5,698 \pm 2,358$	$676,523 \pm 106,958$	0.83
5	2.5 min	1 h	$448,818 \pm 26,010$	$296,360 \pm 38,823$	60.22
10	2.5 min	1 h	$524,235 \pm 73,263$	$318,570 \pm 52,770$	62.20
5	2.5 min	0	$369,737 \pm 82,016$	$340,845 \pm 42,823$	52.03
10	2.5 min	0	$463,636 \pm 55,812$	$398,483 \pm 9,061$	53.34

All vials were immersed rapidly in liquid nitrogen after they had reached -26°C according to the schedule given above. Cells were thawed rapidly to $+37^{\circ}\text{C}$ after about 1 h, medium containing DMSO was removed by centrifugation and each ampoule seeded into one 25 cm^2 culture flask (Falcon Plastics) containing 5 ml DME culture medium. Cell survival was measured as percentage attached (viable) cells compared with floating (non-viable) cells after incubation for 15 h at $+37^{\circ}\text{C}$ (ref. 2). Each figure represents the mean of four independent determinations.

-30°C , and -40°C provided less optimal cooling rates. Larger sample volumes cooled in polypropylene tubes rather than glass recovered less well at -25.6°C , because Farrant *et al.*¹ were working outside the optimal cooling velocity for their particular cells. We have described elsewhere that the cooling velocity can be controlled well by different types of insulation of the vial used for freezing³.

As shown previously^{2,3}, the optimal cooling velocity differs between lymphocytes, red blood cells, Chinese hamster

cells, human diploid fibroblasts and others but the principle is the same.

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DR FARRANT *et al.* REPLY—We believe that the protection of living cells against freezing damage given by cooling rate techniques or by a period at a constant subzero temperature are both due to the effects on the cells of the time of exposure to the subzero conditions.

We have evidence that a continuous reduction in temperature (cooling rate) is not essential for protection. The conclusive point is the onset of protection against subsequent rapid cooling damage with time at a subzero holding temperature once that temperature has been reached. This can be seen (1) in our data¹, where the recovery of lymphocytes and tissue culture cells thawed from -196°C increased with time (between 1 and 10 min) after -26°C had been reached; (2) in other work on increases in protection with time at a constant subzero temperature in spermatozoa^{2,3}, red blood cells⁴, platelets⁵, tissue culture cells⁶, nematodes⁷, and mulberry twigs⁸; (3) from unpublished work with Chinese hamster cells cooled in capillary tubing to -26°C within 17 s, in which protection against rapid cooling to -196°C is absent for the first 1 min and yet increases to a maximum at 2.5 min; and also (4) from the data of Rüdiger *et al.*⁹, where there is an improvement in survival of diploid cells with time at -26°C .

The argument put forward by Rüdiger *et al.*⁹, that cooling rate is essential for protection depends solely on their data showing very low survival of the cells cooled to -26°C within 30 s. It is possible that cooling a 1-ml sample with liquid nitrogen and relying on an 'electric thermometer' of unspecified thermal mass to determine when the sample has reached -26°C (ref. 9), in fact cools regions of the cell suspension to a much lower temperature, thus producing intracellular ice before the period at the holding temperature. This should be checked in

control samples thawed immediately from the holding temperature, which in our experiments gave high survival. Also, with a different cell type, amount of cryoprotective additive (including serum) and the slower thawing rate unavoidable with a larger sample, the optimal conditions of time and holding temperature are different (our unpublished work). The conditions we described originally¹ therefore do not constitute a universal recipe.

In short, cooling rate is important but survival using cooling rate techniques can be explained in terms of the cells being exposed for different times to different subzero temperatures: however, protection acquired with time at a constant subzero temperature cannot be dependent on cooling rate.

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Long-term periodicities in the sunspot cycle

COHEN and LINTZ¹ claim that their work shows the presence of a 179-yr periodicity in sunspot cycles, and that this is a beat phenomenon. Another method of analysis can be used to demonstrate more clearly that a period of approximately this length exists in the data, and is indeed the result of beating between two more rapid oscillations. Cohen and Lintz used the term "beat frequency" in the sense of half

the difference frequency, but here the beat frequency will be taken to be the difference frequency. Whatever definition is adopted, successive maxima in the beats resulting from frequencies with periods 10.9 and 9.7 yr (ref. 1, Fig. 2) occur at 83-yr intervals, taking the frequency separation to be $0.012 \text{ cycle yr}^{-1}$ as they do. The 167-yr interval mentioned by Cohen and Lintz is approximately a waveform repetition period.

To study beat phenomena it is advantageous to consider the transformed sunspot numbers obtained by changing the sign of the numbers for alternate cycles in the way proposed by Bracewell² and others. Cole³ has carried out a spectral analysis on such data by standard methods and the results in his Fig. 1 shows that the resulting power spectrum is much simpler than that obtained by using the ordinary, positive, sunspot numbers. It should therefore be much easier to make deductions about the behaviour of sunspot numbers from this type of spectrum. For instance, as Cole's spectrum (in his Fig. 1d) shows three main peaks, the largest with a period of 22.2 yr and two smaller ones with periods of 19.7 and 18.3 yr, one would expect to find beat frequencies with periods of 175 and 104 yr in the data.

If one uses the ordinary sunspot numbers, any period appearing in the envelope of the maxima will also appear as that of a genuine spectral component, because the envelope of the sunspot minima is almost constant (nearly zero). This need not happen if the transformed sunspot numbers are used, and indeed does not happen in Cole's spectrum. Therefore the 175-yr period in the maxima is certainly a beat phenomenon.

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Matters arising

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DRS COHEN AND LINTZ REPLY—We had hoped that use of the term "beat" would help the casual reader to better understand the phenomena observed. Given the confusion that has arisen, however, we probably should have discussed the sunspot cycle only in terms of the waveform repetition period (or frequency). In doing so, the data indicate that the waveform should repeat about every 179 yr.

Specifically, through correlation analysis, and guided by the periods of significant signatures in the maximum entropy (MESA) spectrum for the data interval 1750-1963, we find that the spectrum of the 12-month smoothed

sunspot numbers shows components at 89.6, 57.1, 11.2, 9.9 and 8.1 yr. We estimate, therefore, that to a first approximation, the repetition period for a waveform produced by the superposition of sinusoids having these periods is about 179 yr ($2 \times 89.6 = 179.2$; $3 \times 57.1 = 171.3$; $16 \times 11.2 = 179.2$; $18 \times 9.9 = 178.2$; $22 \times 8.1 = 178.2$).

That an 89.6-yr component is found in the spectrum suggests that the Sun may be behaving as a nonlinear (square law) device, passing not only the two major driving frequencies (11.2 and 9.9-yr periods), but also generating components corresponding to twice the driving frequencies, and to the sum and difference frequencies. Thus, we might expect to find spectral components at the following frequencies:

$$\begin{aligned} f_2 - f_1 &: 89.6 \text{ yr} \\ f_1 + f_2 &: 5.3 \text{ yr} \\ 2f_1 &: 5.6 \text{ yr} \\ 2f_2 &: 5.0 \text{ yr} \end{aligned}$$

Weak, but significant, components are found in the 214-yr MESA spectrum at periods of 4.8, 5.5 and 5.8 yr (correlation analysis yields period estimates of 4.8, 5.3 and 5.8 yr) and these may represent the expected heterodyne components.

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Population stratification as an explanation of IQ and ABO association

A RECENT study¹ of English villages claims to have shown significant differences in mean IQ between some of the ABO blood group phenotypes. The population can be divided into two groups, namely those who were born locally and those who were born elsewhere, henceforth referred to as locals and non-locals respectively. There is a significant difference in ABO frequencies between the two groups. The frequency of ABO phenotypes varies significantly² throughout England, so that differences in frequencies between the local and non-local populations are not surprising. Also, the non-locals have significantly higher mean IQs than the locals, regardless of the blood group phenotype. The authors¹ claim that "There is thus genetic evidence for at least two population groups in the area, but this alone cannot be responsible for the association between ABO blood group and IQ which the analysis reveals."

We should like to suggest that the observed association could in fact be the result of this stratification of the population. The combination of the difference in ABO frequencies and the difference in

mean IQ between the local and non-local groups causes an association. This is explained below in a very simple model.

The population is divided into two groups, local and non-local. Each group is subdivided into, for example, blood groups, which will be labelled 1, 2, and 3. The population frequencies of blood group i are p_i and q_i for locals and non-locals respectively

$$(i = 1, 2, 3, \sum_{i=1}^3 p_i = 1 = \sum_{i=1}^3 q_i)$$

The total sample size of locals is N_1 and non-locals N_2 . (Table 1). Define $M = N_1/(N_1 + N_2)$.

Denote the mean IQ of locals by x_1 and that of the non-locals by x_2 (independent of blood group). Then the mean IQ of blood group 1, denoted a_1 is given by

$$\begin{aligned} a_1 &= \frac{N_1 p_1 x_1 + N_2 q_1 x_2}{N_1 p_1 + N_2 q_1} \\ &= \frac{M p_1 x_1 + (1-M) q_1 x_2}{M p_1 + (1-M) q_1} \end{aligned}$$

Similarly the mean IQ of blood group 2 is

$$a_2 = \frac{M p_2 x_1 + (1-M) q_2 x_2}{M p_2 + (1-M) q_2}$$

So

$$a_1 - a_2 = \frac{M(1-M)(x_1 - x_2)(p_1 q_2 - q_1 p_2)}{(M p_1 + (1-M) q_1)(M p_2 + (1-M) q_2)}$$

Thus $a_1 \neq a_2$ if $x_1 \neq x_2$ and $p_1 q_2 \neq q_1 p_2$. So if the mean IQ of the locals and non-locals is different and their blood group frequencies are also different, then in the total population the mean IQ of each blood group will be different.

Is this sufficient to explain the difference observed in the Otmoor study? The appropriate parameter values are obtained from Tables 4 and 5 of that paper¹. They are $p_1 = 0.5238$, $q_1 = 0.3869$, $p_2 = 0.3691$, $q_2 = 0.4696$, $p_3 = 0.1071$, $q_3 = 0.1435$, $x_1 = 100.671$, $x_2 = 110.965$. (Blood group 1 refers to the A_1 phenotype and 2 to O.) The values of N_1 and N_2 in those Tables 4 and 5 are different. But the same general result holds for both sets of numbers. The values in Table 4 will be used as they are closest

Table 1 Numbers observed in each subgroup

Blood group	Local	Non-local
1	$N_1 p_1$	$N_2 q_1$
2	$N_1 p_2$	$N_2 q_2$
3	$N_1 p_3$	$N_2 q_3$

to the total used in Table 1. Thus $N_1 = 168$, $N_2 = 460$ so $M = 0.2675$.

Using this model we estimate that the mean IQ of A_1 phenotypes in the total

population sampled would be 107.56 and the mean IQ of O phenotypes 108.67. Thus the expected difference in mean IQ between these two blood groups, for this particular population, is 1.11. (The standard error of this estimate is 0.15 so that the difference predicted is significantly different from zero.) The observed values of 106.95 and 109.75 for A_1 and O respectively are not significantly different from these expected values. Although the observed difference of 2.8 IQ points is significantly different from zero it is not significantly different from the estimated expected value of 1.11. Thus the stratification of the population would be an adequate explanation of the observed differences in IQ between the blood groups.

The model described above does not take account of the observed sex differences in IQ and ABO frequencies, but can be simply extended to do so. In this case the expected difference in IQ between the A_1 and O phenotypes is 1.2 and the same general result applies.

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GIBSON, HARRISON and HIORNS REPLY—We appreciate the model proposed by Thomson and Bodmer and accept that its application to the Otmoor data makes the hypothesis that the association we reported between ABO blood group status and IQ is caused by the presence of two population groups in the area (locally born and non-locally born), more likely than we originally thought. We still however consider that this explanation as we originally stated, is unlikely to be the sole cause for the association on the following grounds.

On the basis of the model, the ability of a test to detect IQ differences between people of blood groups A_1 and O ought to be greatest in those villages where the proportions of locals and non-locals are most equal. There is no relationship between this proportion and the level of association in the various villages. Indeed, in Beckley and Horton, where the sample contained the second highest proportion of locally-born subjects, the

phenomenon was not detectable and the actual differences between A₁ and O were in fact the other way round from those found in the other villages.

Although regional differences in the frequency of ABO genes certainly occur, there is no evidence in the data of Kopec¹ for micro-differentiation between neighbouring Oxfordshire groups from which most of the non-local individuals came. Further the migration matrix analysis of movement into the Otmoor populations² not only predicted that there would be no between-village heterogeneity but that the genetic composition of the villages should be the same as those in the surrounding neighbourhood. It therefore seems unlikely that the differences in ABO frequencies between locals and non-locals arise from their being two primary populations. (We should perhaps add here that further analysis of other polymorphic systems indicates genetic heterogeneity between the Otmoor villages, but this is not shown by the ABO system.)

An overall analysis of the IQ variance according to the ABO status reveals significant heterogeneity. In the four groups, local males, local females, non-local males and non-local females the IQ variance is always smaller for O subjects than for A₁ ones. Although these differences do not reach individual significance, an analysis of variance of logarithms of variances according to blood group status, sex and origin, indicate significant differences by AO status and sex ($t = 2.23$ and 2.03 respectively with 629 d.f.). (This variance analysis includes additional information to that previously reported, but the same trends are present in the original data.) The two-population model cannot account for variance differences but selective migration can. If, for example, high IQ individuals tend to outmigrate from Otmoor, which seems likely, and if these outmigrants tend to have a relatively high frequency of blood group O, then one would find a comparatively low variance of IQ in those O individuals who remained and were tested. Selective migration of this form could also explain the differences in the ABO frequency of the locally and non-locally born groups and particularly, the unusually low frequency of the O gene in the locally born males.

It is perhaps also worth mentioning that in some of the village groups a significant difference in the IQ of A and O people also exists among the non-locals.

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Dantrolene sodium and 'skinned' muscle fibres

HAINAUT and Desmedt¹ have reported the results of their work with Dantrolene sodium (1-[[5-(*p*-nitrophenyl) furfurylidene] amino] hydantoin sodium hydrate salt) and skeletal muscle. In single fibres of frog semitendinosus muscle they showed a greatly reduced twitch force and a shift in the dose-response curve for potassium contractures. These results in general agree with those already obtained with whole muscle². Other workers³ have shown that Dantrolene sodium has no effect on sarcolemma resistance or capacitance, and all evidence²⁻⁴ points to the intracellular Ca²⁺ control mechanisms as the level at which Dantrolene sodium produces its effect.

If the contractile proteins themselves are not involved, the twitch tension following a single release of Ca²⁺ from the sarcoplasmic reticulum may be reduced in two ways: less Ca²⁺ may be released, and also the rate of uptake of Ca²⁺ may be raised. Hainaut and Desmedt¹ show a reduction of 30% in the aequorin luminescence of barnacle fibres, following treatment with high concentrations of Dantrolene sodium, thus demonstrating that Ca²⁺ release is certainly reduced in this muscle. In the frog, however, the twitch may be reduced by 75% with 15 μ M Dantrolene sodium and it is possible that several mechanisms may be involved. A good method for detecting changes in Ca²⁺ uptake into the sarcoplasmic reticulum is the Ca²⁺-gel pipette technique of Gillis⁵, which can be used for frog muscle. Here, controlled local contractions are produced by a short contact with the Ca²⁺-containing gel on an area of the muscle fibre from which the sarcolemma has been mechanically removed. Thus Ca²⁺ is introduced from an external source, and relaxation follows with the uptake of these ions into the sarcoplasmic reticulum. Drops of Dantrolene sodium in aqueous solution were added to the fibre under paraffin oil. The concentration of Dantrolene sodium was about 15 μ M, a saturated solution in 120 mM KCl. In this 'skinned' preparation with no membrane it may be expected that the concentration of the negatively charged Dantrolene ions within the sarcoplasm is much higher than that inside an intact fibre. The volume of Dantrolene sodium solution given was roughly twice the volume of the 'skinned' region of the fibre, so it would be expected that the concentration at the point of the test contractions remained high for some time.

Contraction-relaxation cycles before and after Dantrolene sodium treatment were recorded on cine film and compared for speed and distance of contraction, spread of activation and rate of relaxation.

There was absolutely no change in any of these parameters, either a few seconds after Dantrolene sodium or several minutes later. In view of the undoubted effects of Dantrolene sodium on the intact muscle it would seem that it acts uniquely on Ca²⁺ release—the event bypassed using the Ca²⁺-gel pipette. It is clear that sliding filament reactions and Ca²⁺ uptake are not altered in the 'skinned' fibre. Also, as the spread of activation was not reduced it seems likely that the regenerative release of Ca²⁺ from the reticulum is not inhibited. This aspect of the possible actions of Dantrolene sodium has been discussed previously⁴ in relation to other results.

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¹ Hainaut, K., and Desmedt, J. E., *Nature*, **252**, 728–729 (1974).

² Ellis, K. O., and Carpenter, J. F., *Archs Pharmac.*, **275**, 83–94 (1972).

³ Ellis, K. O., and Bryant, S. H., *Archs Pharmac.*, **274**, 107–109 (1972).

⁴ Putney, J. W., Jr., and Bianchi, C. P., *J. Pharmac.*, **189**, 202–212 (1974).

⁵ Gillis, J. -M., thesis, Louvain, Vander (1972).

DRS HAINAUT AND DESMEDT REPLY—Studies on single muscle fibres have demonstrated that Dantrolene sodium reduces the Ca²⁺ release from the sarcoplasmic reticulum during a twitch¹. The finding that Dantrolene sodium does not accelerate the myoplasmic Ca²⁺ uptake studied in skinned muscle fibres² is in line with our observations on intact barnacle muscle fibres injected with aequorin. Figure 2d–e from our paper¹ indeed showed that the calcium transient, though markedly reduced in size, was not significantly changed in its time course by Dantrolene sodium. The half time of the exponential falling phase of the luminescence transient studied for different depolarisations in six different fibres was 38.9 ms (s.d. = 1.8 ms) before and 38.4 ms (s.d. = 2.9 ms) in presence of Dantrolene sodium. These and other data (K. H., and J. E. D., unpublished) confirm that Dantrolene sodium depresses the Ca²⁺ release but does not affect the rate of reuptake of Ca²⁺ in intact muscle fibres.

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¹ Hainaut, K., and Desmedt, J. E., *Nature*, **252**, 728–729 (1974).

² Brocklehurst, L., *Nature*, **254**, 364 (1975).

¹ Kopec, A. C., *The Distribution of the Blood Groups in the United Kingdom* (Oxford University Press, London, 1970).

² Hioris, R. W., Harrison, G. A., Boyce, A. J., and Kuchemann, C. F., *Ann. hum. Gen.*, **32**, 237–250 (1969).

reviews

A MEASURE of Francis Bacon's influence and importance to the history of ideas lies in the number of ways in which his works can be studied, and the number of books written about him. Not so long ago it was the fashion to emphasise his modernity and see in his writings the nascent scientific revolution of the 17th century. Now there is a useful corrective in a tendency to regard him as a Renaissance man (he was three years older than Shakespeare and Galileo) and to examine his thought in terms of 16th century concepts.

Out of this latter tendency comes Dr Jardine's extremely learned and lively study, based upon a scholarly examination of the dialectical tradition to which Bacon was exposed at Cambridge and which, as Dr Jardine shows, he never entirely shook off. There were in the later 16th century many new treatises on the art of discourse, intended to teach the young student both the art of logical argument and its application in rhetoric. Here we see how Bacon duly followed their precepts in all his works to some extent, most especially in his literary writings and in his logic; it is useful to be reminded what is easy to overlook, that the *Novum Organum* is first and foremost a logical or dialectical work, and only secondarily scientific.

This said, it must be added that this is not the whole of Bacon; this Bacon is studious, scholarly, derivative, pedantic, and eminently forgettable. There is another Bacon, who transcended his

Sides of Bacon



Francis Bacon: Discovery and the Art of Discourse. By Lisa Jardine. Pp. viii+267. (Cambridge University Press: London, 1974.) £4.90; \$15.50.

sources and his dialectical methods of thought to fire the imagination of budding natural philosophers born after (sometimes long after) he wrote. It is revealing to consider Bacon's elaboration of forms in terms of his dialectical education, but it provides neither the whole meaning nor any of the relevance of his work to the advancement of science. Dr Jardine is eminently successful in showing the traditional content; her approach cannot and does not help us to understand why it was important to later natural philosophers that Bacon said of heat that it was "a motion, expansive, restrained, and acting in its strife on the smaller parts of bodies". Nor does it allow us to appreciate that it was relevant to the development of the mechanical philo-

sophy that Bacon connected the "form of whiteness" with the reflectivity of the small particles of bodies. The scientist and historian of science looks favourably upon Bacon's remark: "A leaden ball of a pound weight dropped from a tower reaches the ground in (say) ten seconds: will a ball of two pounds in weight (in which the force of natural motion, as they call it, ought to be doubled) reach the ground in five seconds? No, but it will take almost the same time in falling, and will not be accelerated in proportion to the increase of quantity". Surely this is close to the heart of advancing science in 1623.

It may be true, as Dr Jardine says, that "in treating systematic experimenting as subsidiary to a formal logic which manipulates essential definitions . . . Bacon dismissed as secondary and provisional much of what we now regard as science"; but did he dismiss so much of what was then called natural philosophy? Rather, may it not be the case that his casting of new ideas in an old framework and his use of the language and methods of thought in which his readers in the next two generations were as thoroughly trained as he had been, made these ideas easier to understand, and certainly no less effective?

But modern readers, not so educated, are less able to appreciate the 16th century context of Bacon's thought and, above all, expression. Dr Jardine's book is valuable in providing understanding. **Marie Boas Hall**

THIS volume is the first of a projected series to be based on courses of lectures given at the Harwell Education Centre, by lecturers both from Harwell itself, and from outside. These Harwell lectures have been generally at an introductory level, and the present volume is in line with that trend. This description is in no way to be construed as an implicit criticism, for there has been a clear need for just such a volume. According to the preface, "the principal aim of the book is to provide a balanced and integrated account of instrumental methods (used for the characterisation of materials) for those who carry responsibility for research programmes and who must be able to judge the potential contribution from alternative techniques, and for specialists in any one technique who require an overview in adjacent fields of work".

The book achieves these aims effectively, especially the first; the second category of readers may find some (not all) of the chapters somewhat elementary for their requirements. The book

The stuff tis made of

Modern Physical Techniques in Materials Technology. (UKAEA Research Group—Harwell Series.) Edited by Mulvey, T., and Webster, R. K. Pp. xiv+321. (Oxford University Press: London, November 1974.) £9.50.

is also well pitched for an introductory course in a materials science course at university; I am already using it for that purpose.

Of the 20 chapters (8 by Harwell staff), 4 deal with various forms of

diffraction, 7 with micrographic methods (with a heavy emphasis on electron instruments), 7 on diverse methods of chemical analysis by physical means (optical emission and absorption spectrometry; electron-probe microanalysis; Auger, X-ray and mass spectrometry; activation analysis) and 2 with Mössbauer and magnetic resonance spectroscopy, regarded primarily as means of chemical identification.

Loosely, the book offers advice on how to find out what it looks like and what it's made of. The advances of the last 15 years have changed these twin arts out of all recognition, and this book offers a useful outline of what is now feasible. Some of the techniques are still difficult enough to oblige the wise casual user to contract the work out: Harwell is ready and waiting.

R. W. Cahn

Span Quaternary fields

Pedology, Weathering, and Geomorphological Research. By Peter W. Birkeland. Pp. xiii+285. (Oxford University Press: London and New York, October 1974.) £6.25.

Few books set out to bridge the considerable gaps between the various disciplines in Quaternary studies. This may be a reflection of the range and diversity of material, and consequently of the specialisms demanded of the author. It must, therefore, have required a commendable boldness to embark on a work as complicated as that suggested by the title of Professor Birkeland's new book.

The text spans the interesting and fruitful field between pedology and other Quaternary studies, but each reader will probably have a different view of the likely contents, and may therefore be frustrated by what is actually covered. The title does not represent the emphasis accurately. The major stress lies on the factors of soil formation (chapters 6-11), but there are introductory chapters on soil profiles, properties and classification (chapters 1 and 2), a section on weathering and soil forming processes (chapters 3-5), and a final single chapter on the use of soils in Quaternary studies. Pedologists will be familiar with most of these topics, and anyone interested in weathering and soil processes will find the material rather brief but succinct.

Geomorphologists may be thwarted in their search for appropriate material as there is no chapter specifically upon geomorphological work. Topics of recent geomorphic interest, such as quantitative research into processes, slope analysis and chronology are barely mentioned. The level is more appropriate to senior undergraduate and first year postgraduate students in British universities.

The text is geared to the American market. Nearly all the examples are taken deliberately from North America, and it is a matter of some regret that there are relatively few publications discussed, even in English, from outside that area. The depth of recommended reading varies from 73 references for one chapter down to 11 for another.

The lack of a review of biological factors leads to some imbalance; neither the chapter on vegetation and soil relationships (which fails to consider either fauna or microorganisms), nor the final chapter give adequate consideration to the biotic influences on soil development.

Despite these general criticisms and

a number of minor quibbles, this book makes worthwhile reading on several counts. It does present a summary of recent (particularly American) research and it does bring together the research of diverse but related fields. Amplification of the topics presented, and a more extensive review of allied geomorphological research would add to the value of any future editions.

Peter A. Furley



Unwitting participant: helping scientists to study the ability of penguins to survive in icy wastelands. From *The World Science Year Book, 1975*. Pp. 441. (World Book Encyclopedia Incorporated: Chicago, 1975.) n.p.

Bubble devices

Magnetic Bubbles. By T. H. O'Dell. Pp. x+159. (Macmillan: London and Basingstoke, January 1975.) £8.50.

MAGNETIC BUBBLES will prove invaluable in a variety of roles; it will, for example, provide an excellent introduction and foundation course for device engineers and materials scientists entering the new field of magnetic bubble devices. In spite of the rapidly growing bibliography on magnetic bubbles the extensive references provided by the author will considerably aid established workers in this field to recover previously published information. The author has concentrated on the underlying physics of both the operation of devices and the materials

used. The treatment has emphasised, where appropriate, the mathematical aspects of the subject, and in most cases it has been done in such a way as to inspire confidence, maintaining a balance between the basic philosophy of bubble devices and a rigorous mathematical treatment.

Consider a thin layer of magnetic material which, because of its structure, can easily be magnetised to saturation only in directions normal to itself. A magnetic bubble is a small and mobile cylindrical region, extending through the layer, which is magnetised in a sense opposing the remainder of the layer. Bubbles are used for data processing and storage, each one representing a '1' digit. A bubble device consists simply of an assembly of a number of integrated circuits each of which carries magnetically activated tracks, that is, shift registers, along which are driven patterns of bubbles and gaps representing binary data.

Chapters on magnetostatics and bubble dynamics are particularly well presented, bringing together a well balanced explanation incorporating a number of basic concepts which previously have been scattered among a handful of technical articles. These chapters would be valuable in aiding a first degree course in magnetics.

Two chapters deal with areas close to device design and technology. One provides a review of the range of magnetic materials which have been studied as candidates for bubble devices and explains the important characteristics which ensure adequate bubble stability. Although sufficient detail is given on garnet layers—which are prepared by liquid phase epitaxy—to indicate that they represent a viable device basis the author has omitted details on the epitaxy process itself. That is a pity because were such detail included it would be clear that the models used to represent the layers are idealised in that available layers actually possess nonuniformities in composition throughout their thickness. The other sections dealing with devices provide an excellent treatment of bubble motion in the potential wells produced by the shift register elements. The author's approach is, I believe, unique and is certainly likely to inspire the device engineer.

This review would be incomplete were I not to report the one error I found. Although nothing which follows it is invalidated, the approximation used by the author in Chapter 3 for the exchange constant may mislead readers to assume that the exchange constant is virtually independent of temperature. Actually, it decreases monotonically with increasing temperature, reaching zero at the Curie temperature.

Anthony Marsh

Vertebrate History: Problems in Evolution. (McGraw-Hill Series in Population Biology.) By Barbara J. Stahl. Pp. ix+594. (McGraw-Hill: New York and Maidenhead, 1974.) £8.75.

THIS is the most interesting book on this subject that I have read, because it deals with the problems involved in understanding the course of vertebrate evolution. Most textbooks on vertebrate history are primarily accumulations of information on the structure and diversity of each group of fossil vertebrates, and are really textbooks of palaeo-osteology. Yet, for any reader, the interest of a subject lies in the problems it contains, in the lines of evidence available for solving those problems, and in the methods that can be used for obtaining and analysing that evidence. For example, the problem of the origin of the amphibians is not merely one of osteological modification; the papers that have discussed this subject also include opinions based on studies of the embryology, life history and behaviour of present-day amphibians, the anatomy and physiology of respiration, the ecology, climatology and geography of the Devonian, and the nature and distribution of Devonian sediments. Consideration of such wider topics in turn forces the reader to ponder to what extent they may or may not be relevant to the central question. It provides, therefore, a far better methodological training than the normal textbook description of the trees to the exclusion of the wood they make up.

The book is divided into nine main chapters: fossils (their nature, discovery and investigation); the origin of the vertebrates; bone and cartilage in early vertebrates; the first fishes with jaws; the rise of the modern fishes; the amphibians and their origin; the rise and fall of the reptiles; birds; and finally, mammals. The mammals are thus cut down to their proper status—in most books on vertebrate palaeontology, which give a description of all the diverse orders of mammal, they occupy one third to one half of the text.

The chapters themselves are written in a style that is concise and enjoyable to read, and the concepts and questions that arise are clearly defined. The format is attractive, with topic headings to the side of the text. In considering the main problems raised in these chapters, Dr Stahl has had to read and evaluate a large number of papers. The most important of these are listed in a well-selected bibliography of about 280 titles (some as late as 1972), and there are 216 excellent figures.

Dr Stahl's book cannot replace such textbooks as those of Romer or Colbert as a source of information on verte-

brate palaeontology. It does, however, provide an opportunity for the interested student to find out more without becoming surfeited by facts, and thus to come to appreciate the proper significance of the information those other textbooks provide.

Barry Cox

Evolution



Aepyornis, the Pleistocene elephant bird. From Stahl's new book.

The Genetic Basis of Evolutionary Change. By R. C. Lewontin. Pp. xiii+348. (Columbia University Press: London and New York, 1974.) £2.15.

FOR Darwin, evolution was the conversion of variation among individuals within an interbreeding population into variation between species and genera. The central ideas of molecular biology did not have an immediate impact on evolutionary thought. They did change our concept of mutation from a process of shuttling back and forth between wild-type and mutant states to that of an infinite series of changes which almost never back-tracks. But the full impact waited on the development of two experimental techniques: the determination of amino acid sequences in peptides, and gel electrophoresis. These techniques provided for the first time quantitative answers in genetic terms to two important questions—how fast has evolution proceeded, and how much genetic variation is there in natural populations? The classical evolutionist may object that the level of description has been changed from the important to the trivial—from morphological to biochemical differences.

Dr Lewontin has not only played a large part in the development of theory but was among the first to answer the second question experimentally, in 1966, by showing that in wild populations of *Drosophila* there is probably genetic variation at the majority of loci specifying peptide chains; a finding subsequently confirmed in many species by other workers. This book derives from a series of lectures he gave in Columbia in 1969 and, consequently, perhaps suffers in structure. It is not as wide in scope as the title would suggest and concentrates too much on the evi-

dence from gel-electrophoresis. The approach is historical and the first 100 pages are almost a grand build-up to the presentation of the answer to my second question. The theoretical framework is presented as an antithesis between two views. In the first of these—the 'classical' view—most of the genetic variation in populations is thought to be selectively neutral or harmful. Much evolutionary change may not have been adaptive but has resulted from the chance fixation of neutral alternatives. In the other view—the 'balance' view—it is held that variation is actively maintained in population by selective forces, although there would be disagreement about their exact nature, and that evolution proceeds by the active fixation of available alternatives at loci, with a gradual change of selection pressures over time. The necessary synthesis has not been achieved (though the author clearly leans scientifically and politically on the 'balance' side); Lewontin suggests that the profusion of facts which are now available for the 'theory machine' have merely led to "a great clashing of gears".

There are perhaps two main reasons for the present confusion. The first is that all wild populations have to cope with the wide diversity of environments, both in space and time, and we have neither an adequate description of these, from the point of view of the organism, nor an adequate theoretical treatment of their implications. The second is that differences in fitness between genetic alternatives may be so small (say of the order of 1% or less) that there is great difficulty in measuring them adequately. This is particularly true for the measurement of fertility. With *Drosophila* far too much work has been concentrated on the measurement of viability differences, which is relatively easy to do, even though recent evidence shows that fertility differences are much more important. In my view, the great merit of the book lies in its critical discussion of the present confusion and of the validity, both biological and statistical, of some of the evidence that other workers have put before us.

But we must remember that the loci that we observe with these techniques only refer to a small part of the DNA, the 'single-copy' set coding for proteins. The evolutionists have hardly faced up to the evidence now accumulating from the multiple-copy sequences, such as the satellite DNA or those sequences which code for ribosomal RNA. I suspect that the mechanisms that we shall have to invoke for the evolution of this part of the DNA will lie quite outside our present philosophy and outside the scope of this book.

Alan Robertson

In honour of Lanczos

Studies in Numerical Analysis. (Papers in Honour of Cornelius Lanczos.) Edited by B. K. P. Scaife. Pp. xxii+333. (Academic: London and New York, September 1974. Published for Royal Irish Academy.) £5.00; \$13.00.

THIS Festschrift volume is published under the auspices of the Royal Irish Academy as a tribute to Cornelius Lanczos on the occasion of his 80th birthday (February 2, 1974). It is devoted almost exclusively to numerical analysis, since, although Lanczos made significant contributions to areas of theoretical physics, he is most widely known for his work in this particular field. Professor Lanczos died in Budapest on June 24, 1974 but, happily, he had lived to see this personal and affectionate tribute to him from many distinguished scholars.

Lanczos was educated in Hungary and worked first in Germany (1922–31), then in the United States (1931–54), and lastly in Eire (1954–74), and he was completely imbued and enthused with the traditional viewpoint of the complementary interaction between mathematics and physics, as exemplified by the Göttingen school of mathematics founded by David Hilbert. His work may be divided into two main areas: relativity theory and quantum theory (1922–38) and numerical analysis (1938–74), and in both of these he made important and lasting contributions to knowledge; his abiding interest lay with geometry and the nature of space and time. Lanczos was a prolific author of papers and books, which show clearly his viewpoint of the essential unity of mathematics and physics (the viewpoint, for example, of John von Neumann) and their cultural appeal and importance (as, for example, discussed by Morris Kline).

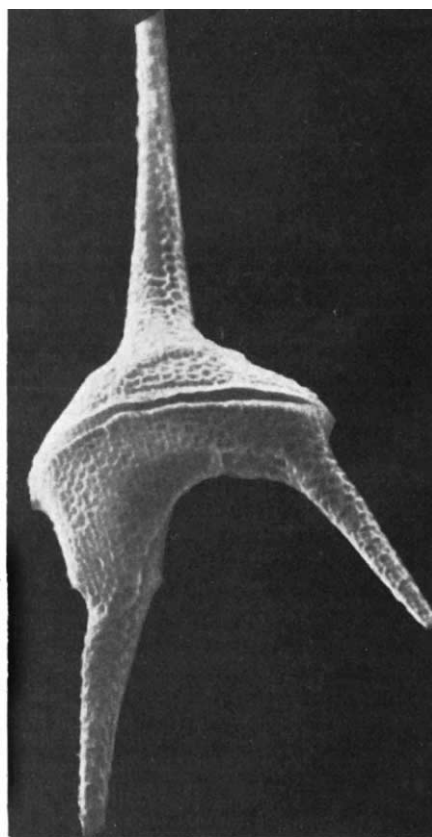
The Festschrift opens with a biographical note on Lanczos, supplemented by a list of publications by him (94 articles and eight books), and it continues with 19 contributed articles. Professor B. K. P. Scaife, who has charmingly dated his editor's foreword for Lanczos' birthday, has obviously sought, by the particular choice of contributors, to associate as far as possible current developments in numerical analysis with Lanczos's original ideas in that field. These include the application of Chebyshev polynomials in approximation theory and the development of the economisation (or telescoping) method and the tau method (1938); the fast-Fourier transform (1942); the first exact method for obtaining all the eigenvectors and eigenvalues of an arbitrary matrix (1951); a fundamental theorem on the

decomposition of an arbitrary matrix (1958); a precision approximation to the Gamma function (1964); and Sigma factors for smoothing Gibbs's oscillations in Fourier series (1966).

In summary, in addition to articles of a specialist nature on topics in numerical analysis, there are three articles of a biographical nature and also a review article on conservation laws in Einstein's general theory of relativity. The book will appeal especially to those with interests in numerical analysis and also to those interested in the life and achievements of Cornelius Lanczos.

H. G. Hopkins

Red tide



Fossil and Living Dinoflagellates. By W. A. S. Sarjeant. Pp. vii+182. (Academic: London and New York, December 1974.) £5.00; \$13.00.

THE gulf called *Sinus arabicus* by the Romans was known to the Arabs themselves as the Red Sea, and it was under that name that it became known to the rest of the civilised world at least as long ago as the ninth century. The visually spectacular effects of the 'red tide' blooms of dinoflagellates have been recognised, therefore, for over 1,000 years, but not until the publication of this work has a book been devoted solely to these extraordinary organisms. Even if there were no other reason, that aspect alone must recom-

mend Dr Sarjeant's volume, but the whole has the additional merits of clarity, readability and profuse, well chosen illustrations. The publishers maintain their high standards with good quality paper, clear printing and format, and strong and attractive binding.

Dr Sarjeant is a palynologist and micropalaeontologist; originally, he intended to deal only with the fossil dinoflagellates and their resting cysts ('hystrichospheres'), and it is with these that his strength lies. Appropriate techniques of sample preparation are outlined clearly and fully, thecate dinoflagellates and cysts are described morphologically, and a separate short chapter is devoted to the taxonomic problems which have resulted from nomenclature based separately upon thecae and cysts. A concise but thorough suprageneric classification of fossil dinoflagellates is appended, and the stratigraphic history of the group is summarised. The acritarchs are mentioned but not described in detail, and there are no illustrations or descriptions of the great majority of the genera and species listed in the stratigraphic section; the reader must turn to the references given by the author for that information. Recent attempts to achieve an objective terminology for the description of cyst walls are not discussed at all. Otherwise, the account is very comprehensive, and it is invaluable to have it (and such a valuable collection of reference lists) now so conveniently to hand.

The chapters dealing with biology, ecology (12 pp.) and palaeoecology (6 pp.) are much less thorough. The faculty possessed by some species to generate extraordinary levels of bioluminescence, with marked periodicity, still deserves further treatment—not only for its own sake, but also for the field work it has stimulated which is contributing greatly to our knowledge of the ecology of these organisms. Much detailed ecological work has been done in recent years, not least in the Red Sea itself, where dinoflagellates dominate primary production for most of the year. The important role played by dinoflagellates in food webs deserves more attention: symbiotic dinoflagellates (zooxanthellae) form a significant primary role in the productivity of coral reefs, and the motile swarms of the 'red tide' itself make their contribution to nutrition as well as to spectacular mass mortalities.

Dr Sarjeant's book will surely deserve a second edition on the merit of its micropalaeontology alone; if neonatology and ecology could then be equally well covered, it would undoubtedly be a major contribution to synoptic studies in the natural sciences.

F. T. Banner

Landscape a l'americaine

The Origin of Landscapes: A Synthesis of Geomorphology. By H. F. Garner. Pp. xx+734. (Oxford University Press: London and New York, October 1974.) £10.75.

THIS long and well-produced text is designed for use at US college level and presumably, therefore, for undergraduates in European universities. In it the author attempts to combine geology, geomorphology, climatology, botany and other branches of natural science in a synthesis of landscape study. The resultant theme seems to be halfway between physical geography in the European sense and physical geology in the American sense.

The presentation of the geomorphic subject matter is said by the author to be "at once new and not new" insofar as it emphasises the relationships of processes and treats any particular process in its several environments: for example, the effect of running water at the surface is discussed in humid, arid, and glacial contexts, and again with respect to alternating environmental conditions. The general theme reflects the genesis of landforms by associations with environmental systems and through the substitution of one system for another in time and space. Thus, the essential theoretical thread that binds together the otherwise detached concepts is the fact that most landscapes are the products of environmental sequence.

The main body of the text opens with a chapter on the concepts of geomorphic theory and the development of geomorphic ideas. The discussion is lively and stimulating but needs some revision. For example, the author thinks the peneplain was the ultimate landform in the Davisian cycle whereas it was penultimate; he considers that downstream increase in the velocity of rivers augments their erosive power whereas, presumably, the velocity is increased because of relative decrease in bed friction or erosional contact.

This introductory matter is strengthened by the succeeding chapter on geomorphic techniques which summarises modern advances. That is followed by a long account, forming nearly one-seventh of the book, on recent tectonic theories and findings, including plate tectonics, mid-oceanic ridges and sea floor spreading. The excellence and detail of this well-illustrated synopsis are not maintained in the next chapter which deals with "surficial geomorphic patterns" and contains *inter alia* a rather elementary account of the atmosphere and of the circulation of the oceans.

The chief superficial landscape sys-

tems are discussed. Humid, vegetated environments—the Davisian norm—are given far less textual space than are glaciation and glacial landforms, arid non-vegetated systems or alternating arid-humid environments. Coasts and mountains are treated separately as polygenetic landscapes, the former being the zone of contact between two distinct environments and the latter the areas within which internal uplift conflicts markedly with external erosion.

The closing chapters consider the application of geomorphological techniques to the theoretical reconstruction of ancient buried landscapes, and the nature of the various physical problems associated with alterations of the present day environment. The volume ends with a glossary of basal geomorphological terms which will be useful for beginners, and a comprehensive index that includes reference to the many striking illustrations, about 600 in all.

An attractive and lavish production, with many progressive ideas, this volume is a welcome and worthwhile addition to educational geomorphological literature. It is not easy, however, for a European to assess it with equanimity. The author thinks that

Great Britain is "an island near the Gulf Stream" and in his index Europe receives 17 entries against 33 for Ecuador, 31 for Venezuela and 25 for Peru. Such a locational bias can be understood as the text is intended for American colleges; but less understandable are the frequent misuse—for depicting spatial distributions—of Mercator's cylindrical world projection (with each pole exaggerated in length 24,000 times to equal the equator), and the occasional textual error, such as Gibraltar, duracrust, mollasse, and cannons (for canons of landscape evolution). Occasionally, too, the language probably becomes rather too-American or too askew to appeal to European taste. Is there arising an Old World-New World linguistic divide? We read that "coastal geomorphic systems . . . exist where the sea embraces the land, lingeringly, and often with what might well pass for passion"; that "the tectonics of plates is a fascinating jigsaw puzzle with many of the nobler attributes of a floating crap game". Whereas the pundits of the Oxford Press seem ignorant of the evils of Mercator, they are obviously experts at crap.

Robert P. Beckinsale

Borers, foulers, sprats, and mangroves

The Biology of Estuaries and Coastal Waters. By E. J. Perkins. Pp. ix+678. (Academic: London and New York, 1974.) £14.60; \$37.35.

PERKINS' attempt to review comprehensively the biology of estuaries and coastal waters has resulted in a large book. The first fifth is introductory and covers the basic physico-chemical and dynamic characteristics of inshore waters; but the treatment is terse and fragmentary. No reader is likely to benefit from such short sections on tides and waves; and the hydrography of estuaries—so fundamental to what follows—surely merits thorough treatment.

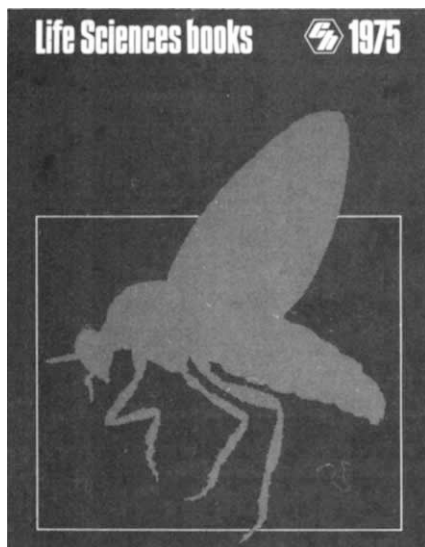
The biota of the various habitats are described in the middle portion of the book, and environmental factors which limit distributions and/or vital activities are discussed. Microorganisms, plants and animals are covered, so most readers will find new information here. There are inconsistencies in approach: discussion is limited largely to estuarine plankton although rocky shores are mainly non-estuarine. And it seems a pity that the ecologically significant differences in the timing of primary production cycles in oceanic and neritic water masses could not have been mentioned. Rocky shore zonation is introduced principally by reference to one British locality but, as coverage (here and throughout the book) is by no means confined to the British Isles, a generalised Stephensonian scheme

would have been more appropriate: the pages on Australian zone-formers may then have integrated better. The superficial approach can be appreciated when it is considered that, although sedimentary beach biota include salt marshes and mangroves the description of this group is restricted to 15 pages.

Even so, the author's interest in the interactions between man and the coastal environment is apparent, and that theme at least is expanded. The detrimental activities of borers and foulers are covered, as is the production of those organisms which man uses for food. Shellfish, except for tropical prawns, are treated fairly fully; but fish, as elsewhere in the book, receive scant attention. Thus the sprat, with an annual catch of up to 100,000 tons in the estuaries and embayments of the North Sea, gets only an incidental mention, and the importance of estuaries as herring and sprat nursery areas is omitted.

The chapter on pollution is somewhat philosophical, but waste disposal and management are both discussed. Prominence is given to the high standards laid down by the United States Federal Water Quality Administration, which contrast with the low standards (except for radioactive waste disposal) acceptable in the UK.

By opting for breadth the author opens himself to criticism for the brevity with which many topics are treated, especially in those fields in



Ultrasonic Communication by Animals

G.D. SALES and J.D. PYE

Hardback : 1974 : 264 pages : illustrated : 412 11920 X : £4.95

The aim of this book is to provide an up to date review of the wide range of acoustic behaviour in animals that is purely or partially ultrasonic. The ultrasonic signals produced by a wide variety of animals are described together with the physiology and biophysics of sound production where this is known. The social significance of the signals is also discussed.

Practical Studies of Animal Development

F.S. BILLETT and A.E. WILD

Hardback : March 1975 : 256 pages : illustrated : 412 10360 5 : £4.80

The aims of this book are to serve both as a practical manual for, and as a general introduction to, the study of animal development. The practicals described vary both in the skills required and the time needed for completion. A special feature is the wide coverage of both invertebrates and vertebrates.

Biological Physics

D.C.S. WHITE

Hardback : January 1975 : 312 pages : illustrated : 412 12650 8 : £6.50

Limp cover : 412 13600 7 : £3.75

Biological Physics provides a lucid introduction to the physical principles normally encountered by undergraduate students taking courses in biology. It differs from many texts with similar titles in that it is basically a *biological* book dealing with physics, rather than a physics textbook without the unnecessary chapters.

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The leaflet shown above describing these and related titles in detail is available from the publishers on request.

which he seems not entirely at home. But the compilation is impressive and uses a vast literature. Unfortunately, though, the bibliography is presented chapter by chapter in the sequence in which they are mentioned, so the location of references is an exasperating business. Mistakes and typographical errors are few and the book is well produced; but the price is far beyond the means of many of those who would wish to buy it.

J. S. Ryland

Life

Life—The Unfinished Experiment. By S. E. Luria. Pp. 167. (Scribner's Sons: New York, June 1973.) \$7.95.

SALVADOR LURIA is well known for his work in virology and molecular genetics. In 1969 he shared the Nobel Prize in Physiology with Delbrück and Hershey. His aim in the present book is to explain modern biology and its social relevance to the general reader.

The scope of Luria's book is superficially the same as Monod's *Chance and Necessity* but there are significant differences. The scientific parts of *Chance and Necessity* were too advanced for the general reader. Luria has attempted a more elementary account. He also deals less with metaphysical issues than Monod, and his social and ethical views are differently slanted. I believe that Luria has succeeded in producing a valuable and readable book, and that it could be usefully incorporated into science courses in schools and generalist courses in universities; and that it would be of interest to laymen and to some professional bioscientists.

Luria emphasises molecular aspects of biology, but not to the exclusion of organismic aspects. He builds up a masterly picture of biology, bringing out key concepts in lively, non-technical ways. Starting with an introduction to evolution theory, he goes on to genes, cells, bioenergetics, form and function in higher organisms, and biogenesis. He concludes by discussing social and philosophical issues connected with biology. All this he compresses into only 150 pages, without losing readability, by pruning out unnecessary detail and dealing only briefly with many of his points. Those with little previous biological knowledge will need supervision or complementary reading to appreciate the more subtle points. An apposite reading list is appended to the text.

Luria's underlying theme is "the dualism of the material nature of life's programme on the one hand and the historical nature of biological evolution on the other hand". In his chapter on evolution theory he first discusses the impact the theory has had on man's image of himself and of nature, and

then goes on to consider the scope and limitations of the theory. He emphasises the mutual interaction of individual and environment, and accepts the 'genetic assimilation' view that selection acts on phenotypes rather than on genotypes. In his chapters on heredity, he introduces the subject in a striking way by comparing modern genetics to the great generalisations of physics. At the same time, he points out the uniquely high degree of order in biological phenomena and its historically-derived nature. In each of the succeeding chapters, he also presents the subjects in stimulating and illuminating ways. He does not try to gloss over difficulties and uncertainties.

In the penultimate chapter, Luria discusses issues of medicine, population and genetic engineering. He considers the humane regulation of population growth to be of overriding importance, but recognises the great social and political problems involved. He discusses possible biological consequences of population control and maintains that eugenics programmes (apart from those meant to reduce serious genetic disorders) should be avoided for both social and biological reasons. Though recognising the great technical obstacles to direct genetic intervention in man (such as genetic engineering and cloning), he considers that "in science, once a task has been clearly defined, its accomplishment is generally only a matter of time", and that some genetic intervention may be in medical use within 30 years. And he explores the social and ethical problems that this would raise in our "competitive, caste-ridden, power-dominated society".

In the final chapter, Luria gives a stimulating glimpse into some philosophical issues relating to biology, such as language and brain structure, nativism and learning, biological and cultural evolution, determinism and free will, values, and existential dilemmas. He emphasises the uniqueness of every human individual. He locates the central dilemma of life science as the relations of human purpose, values and will to the fortuitous nature of the evolutionary process. He considers the human brain to have arisen through a process in which "evolution has dialectically surpassed and denied its own past history". Inevitably, various controversial points arise. Among these, I believe that Luria's treatment of existential problems in terms of natural selection is weak; and I would like to see the physicalist position—which he presupposed throughout—explicated and examined in relation to possible counter-evidence (from, for example, parapsychology). In spite of such criticism, my impression is of a well balanced, humanly sensitive, and penetrating world-view.

Robin Monro

obituary

Roderick O. Redman, FRS, a leading British optical astronomer, has died at the age of 69.

Professor Redman studied mathematics at St John's, Cambridge, gaining his Ph.D. in 1929. At Cambridge, he was greatly influenced by Sir Arthur Eddington, Plumian Professor of Astronomy, and took up his first appointment with the Dominion Astrophysical Observatory at Victoria, British Columbia. He returned to the Cambridge Observatory in 1931 to become its Assistant Director and was elected a fellow of St John's in 1932. In 1937, he became Chief Assistant at the Radcliffe Observatory in Pretoria, South Africa, where he started his researches on astronomical photometry. While in South Africa, he made an expedition to the total solar eclipse of 1940, from which he obtained high dispersion chromospheric spectra superior to those obtained previously. Returning to Cambridge in 1947 as Professor of Astrophysics, he fully integrated the Solar Physics Observatory into a new unit, called the Cambridge Observatories. Under his direction, they rapidly recovered from the difficulties of the war years. His enthusiasm and warmth were his major assets in re-equipping the observatory with good modern instruments. Redman led an equally successful expedition to Khartum in 1952 and served as President of the Royal Astronomical Society from 1959–61. Much of his time before and after his retirement

was devoted to the planning and construction of the 150 inch Anglo-Australian Telescope and the success of this instrument owes much to his enthusiasm. After his retirement, he also took part in the planning of the Northern Hemisphere Observatory.

Anatolii Arkad'evich Blagonravov, academician and one of the leading figures in the Soviet space programme, died on February 4 at the age of 90.

Blagonravov, a Lieutenant-General of Artillery, and a full Member of the Academy of Sciences of the USSR since 1953, became attached to the Soviet space programme during the 1950s as a rocket designer. In 1959, he addressed the press conference held in connection with the launching of Sputnik 1. From 1960 onwards, he published a number of works on space research, including *Physics of Cosmic Space* (1962). He was an early member of the Pugwash movement, Deputy Representative of the Soviet Union at the UN Committee for the Peaceful Use of Space, and Chairman of the Commission of the Academy of Sciences of the USSR on Space Research and Utilisation. He was a full member of the Czechoslovak Academy of Sciences, and a Member of the International Academy of Astronautics, and held a number of Soviet medals and awards, including the Order of Lenin (five times), the Order of the Red Banner of Labour (three times) and Hero of Socialist Labour.

Clarence Ray Carpenter, the psychologist and anthropologist, died in Athens, Georgia, on March 1 at the age of 69.

Dr Carpenter studied monkeys and apes for the clues they gave to human behaviour and began his studies on the social life of monkeys in Central America in the 1930s. In 1937, he was a leader of the Asiatic Primate Expedition to Thailand and Sumatra, and studied gibbons in Siam from the point of view of the evolutionary background of human society. In 1939, he travelled from India with 500 macaques that he resettled on Santiago Island, off the east coast of Puerto Rico, observing them set up home. In 1960, Dr Carpenter took a census of the howler monkeys of Barro Colorado Island, Canal Zone, hoping that a study of their ways would serve as a conceptual bridge between the realms of lower animals and man. From 1940–69, he was on the staff of Pennsylvania State University and became emeritus professor of psychology and anthropology, directing the use of closed-circuit television for educational purposes there. As a leader in promoting educational film and television techniques, he served on the Commission on Instructional Technology and on the Advisory Committee of the US Office of Education, and was a past-president of the Joint Council of Educational Communications and the Association for Higher Education. Dr Carpenter was a former editor of *Behaviour* and of the *Journal of Evolution*.

announcements

Awards

Her Majesty the Queen has approved the grant of a **Royal Charter** to the **Institution of Metallurgists** at a meeting of the Privy Council. **Dr W. E. Duckworth** will be succeeded in May as president by **Sir Montague Finniston, FRS**.

The Zoological Society of London has made the following awards: **Scientific Medal** to **P. F. Baker** (ionic transport across cell membranes) and **H. Kruuk** (behaviour and ecology of gulls and carnivores); **Stamford Raffles Award** to **A. E. Ellis** (study of molluscs); **T. H. Huxley Award** to **I. G. Priede** (circulation during swimming in trout); **Silver**

Medal to **E. Hosking** (animal photography and educational zoology); **Frink Medal** to **J. Z. Young, FRS** (original contributions to wider implications of Zoology).

A. R. Ubbelohde, CBE, FRS, has been awarded the **George Skakol Memorial Award** by the American Carbon Society for contributions to the understanding of the intercalation of graphite by elements and compounds, and of the electrical and thermal properties of graphite.

The Society for Analytical Chemistry has awarded its **Gold Medal** to **R. L. Mitchell** for contributions to emission spectroscopy for trace elements.

International meetings

April 15, **Biochemistry of Blue-Green Bacteria**, Aberystwyth (Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP, UK).

April 16, **Mechanisms of Biocidal and Biostatic Activity**, Aberystwyth (Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP, UK).

April 30–May 3, **Protides of Biological Fluids**, Brugge, Belgium (XXIIIrd Colloquium on Protides of Biological Fluids, Simon Stevin Instituut, Jerusalemstraat 34, B-8,000 Brugge, Belgium).

May 8-12, **Flint**, Maastricht (Mr J. H. M. Nillesen, mesweg 19, Eys-Wittem, The Netherlands).

May 13-16, **International Radiation Protection**, Amsterdam (C. E. Rasmussen, Interuniversitair Reactor Institute, Bergweg 15, Delft, Netherlands).

May 14-15, **Ionic Polymers**, Brunel University (Mrs R. Saunders, Industrial Liaison Bureau, Brunel University, Kingston Lane, Uxbridge UB8 3PH, Middlesex, UK).

May 15-16, **Ethical and Social Questions Posed by 'Engineering' the Human Genome**, New York (Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021).

May 25-28, **Genetic Hazards to Man from Environmental Agents**, Ottawa (Mrs J. Renaud, Room 1-5, Health Protection Building, Health and Welfare Canada, Tunney's Pasture, Ottawa, K1A 0L2, Ontario, Canada).

May 26-30, **Prostaglandins**, Florence (Dr G. C. Folco, Institute of Pharmacology and Pharmacognosy, University of Milan, Via Andrea del Sarto, 21, 20129 Milan, Italy).

May 28-30, **Asthma and Allergy**, Denver, Colorado (Elliott Middleton, Jr, MD, National Asthma Center, 1999 Julian Street, Denver, Colorado 80204).

May 30-31, **Myasthenia gravis**, New York (Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021).

June, **Protein Structure and Evolution**, Prague (Dr Z. Deyl, Czechoslovak Academy of Sciences, Institute of Physiology, Praha 4-KRC, Budejovická 1083, Czechoslovakia).

June 2-5, **Information Transfer in Eukaryotic Cells and Recognition and Responses in Lymphocytes**, Montreal (W. Fred Hink, Ohio State University, College of Biological Sciences, Department of Entomology, 1735 Neil Avenue, Columbus, Ohio 43210).

June 2-6, **Atomic Masses and Fundamental Constants**, Paris (AMCO-5 Secretariat, Institut d'Electronique Fondamentale, Bât. 220, Université Paris-Sud, F-91405 Orsay, France).

June 2-6, **Peptides**, New York (Conference Department, New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021).

June 4-6, **Identification and Role of Cellular Receptor Sites**, Baltimore (Edward G. Bassett, Ph.D., Symposium Coordinator, Miles Laboratories, Inc., Elkhart, IN 46514, USA).

June 7-8, **Biological Monitoring of Water Quality**, Helsinki (Jean-Louis Gaudet, UN Food and Agriculture Organisation, Via delle Terme di Caracalla, 00100-Rome, Italy).

June 9-11, **CO₂ Metabolism and Productivity of Plants**, Madison (Marcia Molldrem, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706).

June 15-18, **Plants and Cancer**, Baltimore (Dr Ralph N. Blomster, Chairman, Department of Pharmacognosy, School of Pharmacy, University of Maryland, 636 W. Lombard St., Baltimore, Maryland 21201).

Person to Person

Golfers. Edinburgh Medical Technicians Golfing Society are organising a 50th anniversary Open Golf Tournament at Broomieknowe Golf Club Midlothian on July 8. Prizes to the value of £200. Open to all Medical and Biological Sciences personnel who hold a national handicap. Entry forms from: David Veitch, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK.

New physical and astronomical theory. Scientist wishes to start international organisation to develop a physical and astronomical theory without Einstein's relativity theory, without the quantum theory and without a postulate or principle which is not proved by a positive experiment within ten years. Those wishing to cooperate, contact: Ir P. A. v. Deinsse, Adm. v. Gentstr. 9, Utrecht, The Netherlands.

Weight-driven laboratory clock. Wanted, on loan or hire, one of those 'retired' regulator clocks, for a programme of experimental and theoretical work on pendulum and escapement errors. Collection and transport arranged, assurance (if required) against damage or permanent alteration (Dr D. M. A. Mercer, Department of Physics, University of Southampton, SO9 5NH, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements, and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Great Britain

Imperial College of Science and Technology. Sixty-seventh Annual Report of the Governing Body, 1973/1974. Pp. vi + 81. Annual Accounts, 1973/1974. Pp. 28. (London: Imperial College of Science and Technology, University of London, 1975.) [241]
Psychoenergetic Systems Vol. 1, Part 1, December 1974. Pp. 1-48. Subscription Rates (per volume of four issues, postpaid): Great Britain: Individuals who warrant that the journal is for their own personal use and who order direct from the publishers, £6; Libraries, Institutions and others £22.75. USA/Elsewhere: Individual subscribers \$19.50/£8.50. Librarian, Institutions, and others \$58/£25. (London and New York: Gordon and Breach, Science Publishers, 1974.) [241]
Neuropathology and Applied Neurobiology, Vol. 1, No. 1, January 1975. Edited by J. B. Cavanagh. (Journal of the British Neuropathological Society.) Pp. 1-110. Published Quarterly. Annual subscription: £14; USA and Canada \$47.50. Single issues: £4; USA and Canada \$13.50 plus postage. (Oxford and London: Blackwell Scientific Publications, 1975.) [271]

Other countries

Environmental Impact Assessment: Principles and Procedures. Edited by R. E. Munn. (SCOPE Workshop on Impact Studies in the Environment (WISE) co-sponsored by United Nations Environmental Programme (UNEP), Environment Canada and UNESCO.) Pp. 160. (Toronto: International Council of Scientific Unions Scientific Committee on Problems of the Environment, 1975.) [131]

New Zealand Meteorological Service. Misc. Pub. No. 109: Meteorological Observations for 1973—Stations in New Zealand and Outlying Islands, including the Cook Group, Tokelau Islands, Niue Island and Western Samoa. Pp. 108. \$1.50. Misc. Pub. No. 110: Rainfall Observations for 1973—Stations in New Zealand and Outlying Islands, including the Cook Group, Tokelau Islands, Niue Island and Western Samoa. Pp. 74. \$1. (Wellington: N.Z. Meteorological Service, 1974.) [131]
 United States Department of the Interior: Geological Survey. Professional Paper 828: Fuller's Earth and Other Industrial Mineral Resources of the Meigs-Attapulgus-Quincy District, Georgia and Florida. By Sam H. Patterson. Pp. 45 + 2 plates. (Washington, DC: Government Printing Office, 1974.) \$2.40.

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 74-20: Reconnaissance Glacial Geology, Northeastern Baffin Island. By D. A. Hodgson and G. M. Haselton. Pp. 10. \$2. Paper 74-25: The Geomorphology of the Swan Hills Area, Alberta. By Denis A. St. Onge. Pp. 6. \$2. Paper 74-61: Stratigraphy of the Amoco IOE A-1 Puffin, B-90 Well, Grand Banks of Newfoundland. By W. A. M. Jenkins, P. Ascoli, L. F. Jansa and G. L. Williams. Pp. 12. \$2. Paper 74-58: List of Translations in the Library of the Geological Survey of Canada, No. 3. Compiled by C. Joanna Christensen. Pp. iv + 43. \$2. (Ottawa: Information Canada, 1974.) [131]

United States Department of the Interior: Geological Survey. Professional Paper 844: Geophysical Investigations of the Pensacola Mountains and Adjacent Glaciated Areas of Antarctica. By John C. Behrendt, John R. Henderson, Laurent Meister and William L. Rambo. Pp. iii + 28 + 2 plates. (Washington, DC: Government Printing Office, 1974.) \$2.05. [151]

Norsk Polarinstitutt. Skrifter Nr. 161: The Billefjorden Fault Zone, Spitsbergen—The Long History of a Major Tectonic Lineament. By W. B. Harland, J. L. Cutbill, P. F. Friend, D. J. Cobbett, D. W. Holliday, P. I. Maton, J. R. Parker and R. H. Wallis. Pp. 72. (Oslo: Norsk Polarinstitutt, 1974.) [151]

United States Department of Agriculture. Index-Catalogue of Medical and Veterinary Zoology. Special Publication No. 3: Ticks and Tickborne Diseases. II. Hosts. Part 1: A-F. Pp. vi + 1-490. Part 2: G-P. Pp. iv + 491-976. Part 3: Q-Z. Pp. vi + 977-1268. By Mildred A. Doss, Marion M. Farr, Katharine F. Roach and George Anastos. (Washington, DC: US Government Printing Office, 1974.) \$12.05 per set of three parts. Sold in sets only.

Seismic Masking of an Underground Nuclear Explosion—Final Technical Report. By Lawrence D. Porter prepared for Air Force Office of Scientific Research, Arlington, Virginia. Pp. xx + 110. (Pasadena, California: Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, 1974.) [171]

Smithsonian Contributions to Zoology, No. 180: Clearwing Moths of Australia and New Zealand (Lepidoptera: Sesidae). By W. Donald Duckworth and Thomas D. Eichlin. Pp. iii + 45. (Washington, DC: Smithsonian Institution Press, 1974.) For sale by US Government Printing Office. \$1.15. [171]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 75-1, Part A: Report of Activities, April to October 1974. Pp. x + 602. (Ottawa: Information Canada, 1975.) \$5. [171]

International Atomic Energy Agency, Vienna. Technical Reports Series, No. 158: Recommended Instrumentation for Uranium and Thorium Exploration. Pp. 93. (Vienna: IAEA; London: HMSO, 1974.) 92 schillings; £2.10; \$5. [201]

National Research Council of Canada. Associate Committee on Scientific Criteria for Environmental Quality—Subcommittee on Pesticides and Related Compounds. Subcommittee Report No. 2: Chlordane—Its Effects on Canadian Ecosystems and Its Chemistry. Pp. 189. (Ottawa: Publications, NRC, 1974.) \$1. [201]
 Australia: Commonwealth Scientific and Industrial Research Organization. The Division of Soils Biennial Report, 1972/1973. Pp. 140. (Melbourne: CSIRO, 1974.) [211]